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EASTMAN

Eastman Chemical Company
P. O. Box 431
Kingsport, Tennessee 37662

8EHQ_0898_14234

July 21, 1998

Document Control Office (7407)
Attn: TSCA Section 8(e)
Room G99 East Tower
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460-0001

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

Ladies and Gentlemen:

Eastman Chemical Company submits the following *final* report as required under TSCA §8(e) for your consideration.

> Methyl Cyclopropanecarboxylate: A four-Week Inhalation Toxicity Study in Rats

If you have questions, you may contact me by telephone at (423) 229-4274 or the technical contact, Karen R. Miller, Ph.D., at (423) 229-1654.

Very truly yours,

F. David Petke

F. David Petke, Ph.D.
Senior Technical Associate
Product Safety and Stewardship

Contains No CBI

cc: 8(e) file

8(e)9807.doc

8EHQ-98-14234

8898 0000201

TSCA HEALTH & SAFETY STUDY COVER SHEET - revised 6/25/96

TSCA CBI STATUS:

☐ **CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)**

Clearly mark the confidential information with bracketing and check the box in the appropriate section (☐ *Contains CBI*). Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

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1.0 SUBMISSION TYPE ☐ *Contains CBI*

Submission date: July 21, 1998

☐ 8(d) X 8(e) ☐ FYI ☐ 4 ☐ Other: specify

☐ Initial submission ☐ Follow-up submission X Final report submission

Previous EPA Submission or Title if Update or Follow-up: Docket Number, if any: # 8EHQ-1097-14050

☐ continuation sheet attached

2.1 SUMMARY/ABSTRACT ATTACHED	**2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID**	**2.3 FOR EPA USE ONLY**
☐ Yes X No	8(e)98-7	

3.0 CHEMICAL/TEST SUBSTANCE IDENTITY ☐ *Contains CBI*

Reported Chemical Name (specify nomenclature if other than CAS name):

CAS #: 2868-37-3

Cyclopropanecarboxylic acid, methyl ester

Purity: >99.9%

X Single Ingredient

☐ Commercial/Technical Grade

☐ Mixture Trade Name: Methyl cyclopropanecarboxylate Common Name: CPCA

	CAS Number	*Name*	*% WEIGHT*
Other chemical(s) present in tested mixture			

☐ continuation sheet attached

8EHQ-98-14234

4.0 REPORT/STUDY TITLE ☐ *Contains CBI*

Methyl Cyclopropanecarboxylate: A Four-Week Inhalation Toxicity Study in the Rat

☐ continuation sheet attached

5.1 STUDY/TSCATS INDEXING TERMS

[CHECK ONE]

HEALTH EFFECTS (HE): X ENVIRONMENTAL EFFECTS (EE): ENVIRONMENTAL FATE (EF):

5.2 STUDY/TSCATS INDEXI NG TERMS (see instructions f or 4-digit codes)

STUDY TYPE: STOX	SUBJECT ORGANISM (HE,EE only): RATS	ROUTE OF EXPOSURE (HE only): INHL	VEHICLE OF EXPOSURE (HE only): AIR
Other:	*Other:*	*Other:*	*Other:*

6.0 REPORT/STUDY INFORMATION ☐ *Contains CBI* ☐ *Study is GLP*

Laboratory: Health and Environment Laboratories, Eastman Kodak Company
1100 Ridgeway Avenue, Rochester, NY 14652

Report/Study Date: June 10, 1998

Source of Data/Study Sponsor (if different than submitter)

Number of Pages: ~~230~~ 360

☐ continuation sheet attached

7.0 SUBMITTER INFORMATION ☐ *Contains CBI*

Submitter: Marc G. Schurger Title: Director, Product Safety and Stewardship Phone: (423) 229-5921

Company Name: Eastman Chemical Company Address: P. O. Box 431, Kingsport TN 37662-5280

Submitter Address (if different):

Technical Contact: Karen R. Miller, Ph.D. Phone: (423) 229-1654

☐ continuation sheet attached

8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS ☐ *Contains CI*

☐ continuation sheet attached

88980000201

Submitter Signature: Marc G Schurger Date: 7/21/98

FINAL REPORT

METHYL CYCLOPROPANECARBOXYLATE
SYNONYM: MCPC

HAEL No.:-97-0208 **EAN: 007777**
CAS No.: 002868-37-3 **PM No.: 15858-00**

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

GUIDELINE

OECD: TG-412
EEC: Annex V., Test B.8

AUTHOR

Lisa G. Bernard, M.S.
Raymond M. David, Ph.D.

TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

LABORATORY PROJECT ID

970208I1

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

STUDY COMPLETION DATE

June 10, 1998

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 97-0201-1 STUDY DIRECTOR: DAVID,R.M. PAGE 1
ACCESSION NUMBER: 007777 05/27/98

STUDY TYPE: BASIC REPEATED INHALATION

M. [signature] 5/27/98
(AUDITOR, QUALITY ASSURANCE UNIT) DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
05/29/97	PROTOCOL SUBMISSION ONE-WEEK STUDY	
06/04/97	CLINICAL SIGNS CHAMBER AIRFLOW/TEMPERATURE/RELATIVE HUMIDITY READINGS CHAMBER CONCENTRATION ANALYSIS 1-WEEK EXPOSURE STUDY	
11/13/97	FINAL REPORT REVIEW 1-WEEK PROBE INCLUDED IN 4-WEEK STUDY	
11/17/97	FINAL REPORT REVIEW INCLUDED IN 28-DAY STUDY REPORT	11/17/97
05/27/98	FINAL REPORT REVIEW INCLUDED IN 28-DAY STUDY REPORT	05/27/98

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 97-0208-1 STUDY DIRECTOR: DAVID,R.M. PAGE 1
ACCESSION NUMBER: 007777 05/27/98

STUDY TYPE: BASIC REPEATED INHALATION (28-DAY)

(AUDITOR, QUALITY ASSURANCE UNIT) 5/27/98 DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY ASSURANCE UNIT. WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES.

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
06/16/97	PROTOCOL SUBMISSION	
06/17/97	CHAMBER AIRFLOW/TEMPERATURE/RELATIVE HUMIDITY READINGS CLINICAL SIGNS DURING DOSE	06/17/97
06/23/97	PROTOCOL AMENDMENT OF 6/23/97 RECEIVED	
07/16/97	BLEEDING-NECROPSY-HEMATOLOGY-CLINICAL CHEMISTRY SPECIMEN COLLECTION SPECIMEN/SAMPLE WEIGHT	07/16/97
10/02/97	GROSS PATHOLOGY HISTOPATHOLOGY PATHOLOGY REPORT ORGAN WEIGHTS	10/06/97
10/06/97	GROSS PATHOLOGY HISTOPATHOLOGY PATHOLOGY REPORT ORGAN WEIGHTS	10/06/97

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 97-0208-1 STUDY DIRECTOR: DAVID,R.M.
ACCESSION NUMBER: 007777

10/06/97	RECORDS REVIEW HEMATOLOGY CHAMBER AIRFLOW/TEMPERATURE/RELATIVE HUMIDITY READINGS CLINICAL CHEMISTRY CELL MORPHOLOGY NOMINAL CONCENTRATION DURING DOSE CLINICAL SIGNS	10/07/97
10/16/97	RECORDS REVIEW APPENDIX - SUMMARIES	
11/03/97	RECORDS REVIEW CONCENTRATION DETERMINATION	11/03/97
11/13/97	FINAL REPORT REVIEW INCLUDES PROBE STUDY 97-0201	
11/17/97	FINAL REPORT REVIEW ALSO INCLUDES 1-WEEK PROBE	11/17/97
05/27/98	FINAL REPORT REVIEW ALSO INCLUDES 1-WEEK PROBE	05/27/98

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

United States Environmental Protection Agency, Toxic Substances Control Act, Good Laboratory Practice Standards, 40 CFR Part 792;

Annex 2, Organisation for Economic Cooperation and Development, Guidelines for Testing of Chemicals [C(81)30(Final)].

Raymond M. David, Ph.D.
Study Director

6/10/98
Month/Day/Year

Karen R. Miller, Ph.D.
Sponsor's Representative

6/9/98
Month/Day/Year

SIGNATURE PAGE

Lisa G. Bernard, M.S.
Report Author

5-27-98
Month/Day/Year

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Study Director, Report Author

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Director, Health and Environment Laboratories

5/20/98
Month/Day/Year

Karen R. Miller, Ph.D.
Sponsor's Representative

4/9/98
Month/Day/Year

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ABSTRACT

METHYL CYCLOPROPANECARBOXYLATE
SYNONYM: MCPC

HAEL No.: 97-0208 **EAN: 007777**
CAS No.: 002868-37-3 **PM No.: 15858-00**

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

Groups of five male and five female Sprague-Dawley rats were exposed to target vapor concentrations of 0.0, 0.1, 0.5, or 1.0 mg/L of the test substance 6 hours per day, 5 days per week, excluding holidays (four days per week during weeks with holidays), for 21 exposures. The mean daily time-weighted average (MIRAN®) concentrations for all exposure group and the mean weekly analytical (GC/FID) concentrations for the mid- and high-concentration group were within 10% of the target concentrations. The mean weekly analytical (GC/FID) concentration for the low-concentration group was 26% higher than the target concentration. The temperature and relative humidity inside the chambers during exposure were 19.2 - 21.8°C and 51 - 78%, respectively.

All animals survived to study termination. Animals were observed for clinical signs of toxicity prior to exposure, once per hour during exposure, and 30 minutes to one hour after exposure. Animals exposed to 1.0 mg/L exhibited transient reduced activity levels of minimal severity during exposure on Days 7-29. The 0.1 and 0.5 mg/L animals appeared normal during exposure. Following the exposure period, some animals from the 0.5 and 1.0 mg/L male groups and from all female groups exposed to the test substance had porphyrin nasal discharges or dried porphyrin stains around the nose, with the frequency being much higher for the female rats. Porphyrin discharges or stains were seen less frequently during the morning examination before exposure. Additionally, reduced feces were observed for the 0.5 mg/L female groups and 1.0 mg/L male and female groups. All other observations were considered to be incidental to treatment. Body weights and feed consumption were measured at least weekly. Mean feed consumption was lower ($p \leq 0.05$) for the 1.0 mg/L male group on Day 7 when compared with the control group. No other differences in mean feed consumption were observed. Mean body weights for all exposure groups were comparable with those of the control group throughout the study.

At study termination, animals were anesthetized with Metofane™ and blood was obtained from the posterior vena cava for clinical chemistry and hematology analyses. Fasted body weight and selected organ weights were measured at necropsy. Selected tissues were collected from all animals. All tissues collected from the 0.0 and 1.0 mg/L groups were examined microscopically. In addition, tissues from potential target organs were examined for the 0.1 and 0.5 mg/L groups. No toxicologically significant changes were observed for hematology parameters. Serum clinical chemistry changes consisted of higher ($p \leq 0.05$) mean urea nitrogen levels for the 0.5 and 1.0 mg/L male groups and for all test substance exposed female groups, higher ($p \leq 0.05$) mean

glucose levels for 0.5 and 1.0 mg/L female groups, and higher ($p \leq 0.05$) mean potassium levels were for all exposed female groups when compared with the control groups. Changes in mean sodium levels and in mean albumin/globulin ratio were also observed, but were not considered biologically significant. All other clinical chemistry parameters were comparable among the groups.

Mean relative heart weights were higher ($p \leq 0.05$) for the 0.5 and 1.0 mg/L male groups and for all exposed female groups when compared with the control group. The mean relative liver weight was higher ($p \leq 0.05$) for the 1.0 mg/L male group, and mean absolute and relative liver weights were higher ($p \leq 0.05$) in a concentration-dependent manner for all exposed female groups when compared with the control group. Mean absolute and relative adrenal gland weights and the mean absolute epididymides weights were lower ($p \leq 0.05$) for the 1.0 mg/L male group when compared with the control group. The mean absolute kidney weight was higher ($p \leq 0.05$) for the 0.1 mg/L female group, and the relative kidney weights were higher ($p \leq 0.05$) for all exposed female groups when compared with the control group. No other exposure-related terminal body weight or organ weight differences were observed.

When the tissues were examined histopathologically, exposure-related effects were observed in the heart, liver, testes, and epididymides. Heart effects consisted of myocyte vacuolation, myocarditis, and muscle fiber degeneration for all exposed male and female groups. Liver effects consisted of hepatocellular cytoplasmic vacuolation for all male and female test substance exposed groups; these changes may be an adaptive response to exposure to the test substance since there were no indications of hepatocellular damage from the clinical chemistry data. Testicular and epididymal effects consisted of spermatid and/or spermatozoa degeneration for the 1.0 mg/L male group. Additionally, slightly greater than expected degrees of cytoplasmic vacuolation were observed in the renal tubules for some of the 1.0 mg/L female rats and a mild decrease in cellularity with in the sternal bone marrow was observed for three 1.0 mg/L female rats and one 0.5 mg/L female rat, as well as a minimal decrease in cellularity within the sternal bone marrow of one 0.1 mg/L female rat.

Based on the heart lesions which were observed at 0.1 mg/L, the lowest concentration tested, a no-observed-effect concentration (NOEC) was not determined. In addition, the effect on the heart was considered to be adverse. Thus, a no-observed-adverse-effect concentration (NOAEC) was not identified. Effects on the liver which were considered adaptive, and were not considered to constitute an adverse effect.

STUDY AND TEST SUBSTANCE INFORMATION

Testing Facility

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

Project Participants

Study Director:	Raymond M. David, Ph.D.
Toxicologist:	Lisa G. Bernard, M.S.
Study Technician	Reade A. Moulton
Hematologist/Clinical Chemist:	Robert. E. Emmons, B.S.
Necropsy Pathologist/Veterinarian:	Milan S. Vlaovic, D.V.M., Ph.D.
Histopathologist:	Robert H. Garman, D.V.M., DACVP, Consultants in Veterinary Pathology
Analytic Chemist:	Nancy Porter, B.S.

Sponsor

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

Sponsor's Representative: Karen R. Miller, Ph.D.

Test Substance Characterization

Test Substance Name:	Methyl cyclopropanecarboxylate
Synonym:	MCPC
HAEL No.:	97-0208
EAN:	007777
CAS No.:	002868-37-3
PM No.:	15858-00
Lot No.:	X25234-116-1
Physical State and Appearance:	Liquid, Colorless
Source of Test Substance:	Eastman Chemical Company, Kingsport, TN
Laboratory Project ID:	97020811

Study Dates

Study Initiation Date:	June 11, 1997
Experimental Start Date:	June 16, 1997
Experimental Completion Date:	May 15, 1998

Purity, Structure Confirmation, and Stability Determination

The purity of the test substance was determined by gas chromatography with flame ionization detection (GC/FID) to be ≥99.9% prior to use on the study and ≥99.9% at study termination. Based on these data, the test substance was considered to be stable during the test period. The structure of the test substance was confirmed using mass spectrometry. The mass spectrum of the test substance was consistent with published spectra for this substance. The analytical report for purity analysis and structural confirmation is provided in the Appendix beginning on page 173.

PURPOSE

The purpose of this study was to evaluate the sub-acute effects of the test substance in the rats following repeated inhalation exposures for four weeks.

MATERIALS AND METHODS

Test System

Five male and five female Sprague-Dawley® rats [SAS:VAF®(SD)] obtained from Sasco, Inc. Kingston (Stone Ridge, NY) were randomly assigned to each exposure group. The male and female rats were 48 days of age and weighed 200 ± 8 (male) or 165 ± 7 (female) grams (mean ± SD), respectively, at the start of the study. Rats were chosen for this study because they are a common representative species for inhalation toxicity studies. Also, the rat is the rodent species recommended for use in the Organisation for Economic Cooperation and Development (OECD) and European Economic Community (EEC) Test Guidelines.

Husbandry

Housing

Animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited vivarium in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). During nonexposure periods, rats were singly housed in stainless-steel, wire-mesh cages in a room separate from the exposure room. No other study was housed in the same room as this study. Exposure cages were washed daily. Housing cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed daily.

Environmental Conditions

The study room was maintained at 20 - 23°C and 48 - 61% relative humidity. A photoperiod of 12 hours light from 6 a.m. to 6 p.m. was maintained.

Acclimation Period

The animals were isolated upon arrival and allowed to acclimate for a period of five days. Animals were judged to be healthy prior to testing and were released for testing by the Staff Veterinarian.

Feed

Certified Rodent Diet (PMI® #5002, meal) was available *ad libitum* except during exposure. Feed containers were cleaned and refilled at least once a week. No known contaminants which would interfere with the outcome of this study were present in the feed. Analyses of feed are maintained on file within the testing laboratory.

Water

Water was available *ad libitum*, except during exposure, through an automatic watering system. The source of the water was the local public water system. There have been no contaminants identified in periodic water analyses that would be expected to interfere with the conduct of the study. Semiannual analyses of water are maintained on file within the testing laboratory.

Experimental Design

Identification

Upon arrival, all rats were identified by uniquely-numbered metal ear tags. During randomization, study-specific animal numbers were assigned to each animal. Cage cards, color-coded for each group, contained the study-specific animal number and the ear tag number.

Randomization

The test animals were culled from the stock population based on body weight and were randomly assigned to groups using computer-generated lists. The body weights of individual animals in the culled population did not exceed 20% of the mean for each sex. Following randomization, the body weights of all groups were compared by analysis of variance to insure that there were no statistically significant differences prior to initiation of exposure.

Test Procedures

This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals: Guideline 412, Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (Adopted May 12, 1981) and European Economic Community (EEC), Annex V., Test B.8. Repeated Dose (28 Days) Toxicity (Inhalation) as required by Council Directive 92/69/EEC of July 31, 1992.

Selection of Exposure Concentrations

Exposure concentrations were selected by the Sponsor based on test results of a one-week probe study during which five male and five female rats per group were exposed to 0.0, 1.0, 2.0, or 3.0 mg/L of the test substance 6 hours/day for up to five days. All exposure conditions were as described below. Based on mortality and /or moribundity at 2.0 and 3.0 mg/L, test substance concentrations of 0.0, 0.1, 0.5, and 1.0 mg/L were selected for the four-week study. The data collected during the one-week probe study are presented in the Appendix.

Experimental Design, continued

Exposure

The inhalation exposures were conducted in 590 L stainless-steel and glass inhalation chambers at target vapor concentrations of 0.0, 0.1, 0.5, and 1.0 mg/L. Animals were singly housed during the 6-hour exposures. A diagram of the chamber and placement of cages within the chamber is provided in the Appendix. The animals were moved sequentially each day to new cage positions within the chamber. Cage positions 10 through 19 were used for this study. The exposure chambers were maintained under negative pressure relative to room air. The air flow, temperature, and humidity were recorded every 30 minutes. Chamber vapor concentrations were recorded at least once each hour.

Exposure Atmosphere Generation

The test atmosphere was generated by metering the test substance into glass distillation columns packed with glass beads. Filtered, compressed air was passed through the glass bead-packed columns to evaporate the test substance. The test substance delivery rate and air flow rate were adjusted to produce the desired chamber target vapor concentration. The resultant vapor was directed via glass tubing to a tee just upstream of the inhalation chamber where it was mixed with filtered, conditioned outside air to produce a total airflow of 121 to 172 Lpm (12 to 17 air changes per hour). A diagram of the generation system is provided in the Appendix. A Micro Laser Particle Counter (model μLPC-301, Particle Measuring Systems, Inc., Boulder, CO) was used to measure the number and size of particulates in the chamber. The results indicated that an aerosol of the test substance was not present.

Weekly Vapor Concentration Determination

Once each week (Days 1, 8, 15, 22, and 29), samples of chamber test atmosphere were collected into Tedlar bags. These samples were analyzed using a GC/FID. The results of these analyses are reported in the Appendix beginning on page 194.

Daily Vapor Concentration Determination

Chamber vapor concentrations were monitored with a multipositional air sampling and analysis system. The system consisted of a single MIRAN® IA infrared gas analyzer (Wilks Foxboro Analytical, South Norwalk, CT) and a computer-operated four-port sampling valve (Valco Instruments, Houston, TX).

Chamber vapor samples were continuously collected from each chamber through TEFLON® tubing (0.48 mm i.d.). The valve position was periodically changed to sample

Experimental Design, continued

Daily Vapor Concentration Determination, continued

from each chamber at least once each hour. The voltage output of the MIRAN® and chamber concentration were printed in real-time and captured on electronic media. Voltage data were converted to concentration by linear interpolation between the calibration data points immediately on each side of the sampled data.

A time-weighted average exposure concentration was calculated using the following formula:

$$TWA = \frac{\Sigma\{[T_2 - T_1][(C_1 + C_2)/2]\}}{\Sigma(T_2 - T_1)}$$

where: TWA = time-weighted average exposure concentration (mg/L)
T_1 = the earlier time from each consecutive concentration determination (increment from 1 to n-1)
T_2 = the later time from each consecutive concentration determination (increment from 2 to n)
C_1 = the concentration at time T_1
C_2 = the concentration at time T_2

MIRAN® IA Infrared Analyzer Operating Parameters And Calibration

Chamber distribution and the Day 0 and 1 test atmospheres were monitored using one Miran® (Cell #4935). Due to technical difficulty with this Miran, a second Miran® (Cell #2075) was calibrated and placed in service on Day 1. The second Miran® was used for the remainder of the study.

The infrared analyzer operating parameters were as follows:

	Chamber Distribution and Days 0-1	Days 1-29
MIRAN® No.	3	1
Cell No.	4935	2075
Pathlength (m)	5.25	11.75
Wavelength (μm)	3.30	3.30
Slit width (mm)	1	1
Response Time (sec)	4	4
Range (Absorption)	0.25A	1A
Gain	x10	x10
Cell Temperature (°C)	25	25
Cell Pressure (atm)	0.833	0.833
Cell Volume (L)	5.64	5.64

The wavelength used for monitoring concentration was selected based on a comparison of infrared spectra of the test substance to that of air.

Experimental Design, continued

MIRAN® IA Infrared Analyzer Operating Parameters And Calibration

The infrared analyzer was calibrated by making serial injections (Hamilton microliter syringe) of the test substance into a closed-loop cell. The concentration was determined using the following formula:

$$C = \frac{(V_1)(\rho)}{(V_2)(P)}$$

where: C = concentration (mg/L)
V_1 = Injection volume (µL)
ρ = test substance density (g/mL)
V_2 = MIRAN® cell volume (5.64 L/1 atm)
P = Cell pressure (atm)

Three sets of serial injections were made to produce a mean calibration curve of test substance concentration versus infrared analyzer output voltage.

An infrared analyzer calibration check was performed just prior to each exposure by injecting a measured amount of the test substance into the MIRAN® closed loop. The infrared analyzer output voltage was converted to test substance concentration and compared with the calculated expected concentration. If the variation of the calibration concentrations were within 10% of that expected, the calibration was accepted .

Nominal Concentration Determination

The nominal concentration was calculated by dividing the amount of test substance consumed from the reservoir (determined gravimetrically) by the total chamber air flow using the formula:

$$NC = \frac{(G)(C)}{(V)(T)}$$

where: NC = Nominal concentration (mg/L)
G = Amount of test substance vaporized (grams)
C = Conversion from g to mg
V = Mean chamber air flow (Lpm)
T = Length of exposure (minutes)

Experimental Design, continued

Chamber Vapor Homogeneity

A test to determine variations in concentration at different positions within the exposure chambers was conducted prior to study initiation. The air from the breathing zones of cage positions 10, 12, 14, 16, 18, and 19 was sampled as described under Vapor Concentration Determination and compared with the concentration at a fixed reference position (cage 15). Based on deviations from the reference position of less than 12%, the chamber atmosphere was considered to be homogeneous.

Air Flow Measurement

Total chamber air flow was a combination of compressed air, which was used to vaporize the test substance and to carry the vapor from the generation system to the inhalation chamber, and dilution air. The compressed air flow rate was continuously monitored using a flowmeter. The dilution air flow was adjusted and monitored throughout the exposure using an Omega Air Velocity Transducer (FMA-602-V-S) and Ratemeter (DPF66-RS232). The dilution air flow rate was calculated using the following formula:

$$Q = \frac{(A)(V)}{1000}$$

where: Q = Supply air flow rate (Lpm)
A = Cross sectional area of the dilution air duct (cm^2) [πr^2, r = 1 in.]
V = Supply air linear velocity (cm/min.) [air velocity meter readings are in ft./min.]
1000 = Conversion Factor

Oxygen Level

The oxygen content of the chamber exposure atmosphere was measured during exposure from the reference position using an Model K Oxygen Indicator (Johnson-Williams Products, Bacharach Instrument Co., Mountain View, CA). The oxygen content of the chamber exposure atmosphere was ≥ 20%.

Chamber Temperature and Humidity

Chamber temperature and humidity were measured using wet/dry bulb hygrometers and were recorded twice each hour during exposure.

Experimental Design, continued

Disposition of Groups

Animals were distributed into groups as follows:

Group	Exposure Concentrations	Number of Animals	Animal Numbers	
			Males	Females
1	Control / 0.0 mg/L	5 Males & 5 Females	501 - 505	521 - 525
2	Low / 0.1 mg/L	5 Males & 5 Females	506 - 510	526 - 530
3	Mid / 0.5 mg/L	5 Males & 5 Females	511 - 515	531 - 535
4	High / 1.0 mg/L	5 Males & 5 Females	516 - 520	536 - 540

Animals were exposed 6 hours per day for five days per week (Monday to Friday), excluding holidays (four days per week during the week of July 4th), for four consecutive weeks, and for an additional two days (Monday and Tuesday) of the fifth week. All surviving animals were euthanatized and necropsied on the day following the last exposure.

Clinical Observations

Rats visible through chamber windows were observed for clinical signs during exposure. Tapping sounds were made on the outside of the chamber with a key or other metal object to assess the animals' activity level. Before and after exposure, each rat was removed from its cage and examined. Cageside observations were conducted once a day on weekends and holidays. Observations included, but were not limited to, examination of the hair, skin, eyes and mucous membranes, motor activity, feces, urine, respiratory system, circulatory system, autonomic nervous system, central nervous system, and behavior patterns.

Body Weight and Feed Consumption Determinations

Body weights were measured, prior to exposure, on Days 0, 7, 14, 21, and 28. Feeders were weighed on Days 7, 14, 21, and 28. Animals were fasted the day prior to necropsy. Terminal body weights were measured after exsanguination, but prior to necropsy.

Experimental Design, continued

Blood Collection and Euthanasia

Animals were fasted overnight beginning after the last exposure. The following day, animals were anesthetized with Metofane™ and blood was collected from the posterior vena cava. The blood was placed into vacutainer tubes and allowed to clot for analyses of serum. Other tubes containing an anticoagulant were used for analyses of whole blood samples. Blood smears were also prepared for blood cell counts. Following blood collection, the animals were euthanatized by exsanguination. Animals were bled and euthanatized in a random order based on a computer-generated list.

Hematology and Clinical Chemistry Examinations

Clinical pathology assays were conducted using a Roche Analytical Instruments Cobas Fara II serum chemistry analyzer, Technicon H•1 System hematology analyzer, Helena Laboratories Titan Gel Electrophoresis System (A/G ratio and albumin), BBL Fibrosystems (prothrombin times), and Corning Flame 480 for sodium and potassium. Hematology tests included: hemoglobin concentration, hematocrit, red blood cell count, white blood cell count, red blood cell indices, prothrombin time, and platelet count. Slides containing blood smears were examined for cellular morphology and differential white blood cell count. Clinical chemistry tests included: alanine aminotransferase, sorbitol dehydrogenase, creatinine, urea nitrogen, glucose, total bilirubin, total protein, albumin, albumin/globulin ratio, calcium, phosphorus, sodium, potassium, cholesterol, and triglycerides.

Necropsy

Following exsanguination, the animals were weighed and necropsied. The following tissues were fixed in 10% buffered formalin: nasal passages, trachea, lungs, larynx, heart, stomach, duodenum, jejunum, ileum, cecum, colon, liver, salivary glands, kidneys, urinary bladder, adrenal glands, thyroid glands, thymus, spleen, mesenteric lymph nodes, cervical lymph node, sternum (with bone marrow), brain, cervical spinal cord, sciatic nerve, testes, epididymides, male accessory sex glands, ovaries, vagina, uterus, and Fallopian tubes.

Organ Weights

The lungs, liver, kidneys, spleen, thymus, adrenals, heart, brain, epididymides, and testes were weighed. Paired organs were weighed together.

Experimental Design, continued

Histopathology

For the control and high-dose groups, all tissues were embedded in paraffin and sectioned at 4 µm, except for the brain which was sectioned at 5 µm. The resulting tissue sections were stained with hematoxylin and eosin (H&E) stains and examined for histopathology. For the mid- and low-concentration groups, sections of the liver, heart, testes, and epididymides for male rats and liver, heart, and bone marrow for female rats were prepared in a like manner and examined microscopically.

Data Storage

The final report, tissues, paraffin blocks, slides, data sheets, all nonperishable raw data, and an aliquot of the test substance have been stored in the testing facility archive managed under GLP-mandated conditions.

Calculations and Statistical Procedures

Mean values were calculated for time-weighted average atmospheric concentration, chamber temperature, chamber relative humidity, nominal concentration, body weight, body weight change, feed consumption, serum chemistries, hematology values, organ weights, and organ-to-body weight ratios. Body weight, body weight change, hematology values, clinical chemistry data, organ weights, and organ-to-body weight ratios were evaluated using the following computer-generated statistical tests: Bartlett's test ($p \leq 0.01$), one-way analysis of variance (ANOVA) ($p \leq 0.05$), and Duncan's multiple range test ($p \leq 0.05$) or Dunnett's t-test (body weight change only) ($p \leq 0.05$) to indicate statistical significance.

When the variances of the means were not considered equal by the Bartlett's test ($p \leq 0.01$), the data were evaluated using a Kruskal-Wallis H-test and Mann-Whitney U-test.

Protocol and Standard Operating Procedure Deviations

On Day 1, vapor concentrations were not monitored using a Miran IA infrared analyzer for the first 4 hours of exposure due to technical difficulty with the Miran. A second Miran was calibrated and placed in service. Grab samples were collected and analyzed using gas chromatography; these values indicated that the exposure concentrations were at least at target levels until the second Miran could be calibrated. This deviation did not impact the outcome of the study.

The Daily Environmental Record (i.e. temperature and humidity readings) was not completed for the housing room on July 4. On July 5, the minimum-maximum thermometer indicated a temperature range of 21-22°C. Therefore, the temperature on July 4 was within the range specified in the protocol. According to the electronic monitoring system (a system which was recently installed and calibrated, but not yet fully validated), the relative humidity in the housing room was 40.8-45.8% with a mean of 43.2% on July 4. Additionally, the relative humidity in the adjacent rooms which share a common air supply was 49% and 54% on July 4. This deviation did not impact the outcome of the study.

At necropsy, a terminal body weight was not collected for Rat 526. This deviation had minimal impact on the study; relative organ to body weight ratios for this 0.1 mg/L animal were not calculated.

No other SOP or protocol deviations occurred during the study.

RESULTS

Probe Study

For selection of exposure concentrations, groups of five male and five female Sprague-Dawley rats were exposed to target vapor concentrations of 0, 1, 2, or 3 mg/L of the test substance 6 hours per day for five consecutive days. The animals were euthanatized on Day 7.

All 3 mg/L male and female rats were euthanatized *in extremis* prior to exposure on Day 2. For the 2 mg/L group, one male rat (#415) was euthanatized *in extremis* following exposure on Day 3, one female rat (#433) was found dead on Day 3 (prior to exposure), three female rats were euthanatized *in extremis* following exposure on Day 3, and the fifth female rat was euthanatized *in extremis* prior to exposure on Day 4. No mortality was observed for the 1 mg/L group.

During exposure, reduced activity was observed in a concentration-dependent manner. The 3 and 2 mg/L rats exhibited reduced feces, reduced activity, gait abnormalities (wobbly gait, hypotonic gait, limping), dyspnea, rapid, shallow respiration, hypothermia, dehydration, partially closed eyes, excessive tearing, softened feces, haircoats which were wet or stained with urine or feces, and/or porphyrin discharges or stains around the nose or eyes. Tremors were observed on Day 1 for three 3 mg/L female rats (#436, #437, and #438), on Day 2 for three 2 mg/L male rats (#411, #413, and #415), and on Days 2 and/or 3 for three 2 mg/L female rats (#431, #432, and #435); two of the male 2 mg/L rats (#411 and #413) which exhibited tremors survived to study termination. On Days 6 and 7, the surviving male 2 mg/L rats appeared normal. One 1 mg/L male rat appeared normal during non-exposure periods. The other nine 1 mg/L animals exhibited reduced feces, dehydration, softened feces, haircoats which were wet or stained with urine, and/or dried porphyrin stains around the nose. The 0 mg/L male and female rats appeared normal throughout the study.

Mean feed consumption and mean body weights were lower ($p \leq 0.05$) on Day 2 for the 2 and 3 mg/L male and female rats when compared with their respective control group, with mean body weight changes of -19% to -24%. The surviving 2 mg/L male rats gained weight after Day 2, resulting in a 7% greater mean weight on Day 7 when compared with their initial mean body weight. Mean feed consumption was lower ($p \leq 0.05$) for male and female 1 mg/L rats and mean body weights were lower ($p \leq 0.05$) for the female 1 mg/L rats on Day 2 when compared with the control group, resulting in mean body weight changes of -6% (male) or -16% (female). All 1 mg/L rats gained weight between Days 2 and 7, resulting in a 11% (male) or 8% (female) greater mean body weight when compared with their initial mean body weight.

Based on these results, concentrations of 1.0, 0.5, 0.1, and 0.0 mg/L were selected by the Sponsor's representative for this study.

Exposure Conditions

A summary of exposure conditions is presented in the summary tables on page 32. The mean weekly analytical (GC/FID) concentrations (± standard deviation), of the test substance in air test atmospheres were 0.126 ± 0.015, 0.546 ± 0.019, and 0.970 ± 0.100 mg/L compared with target concentrations of 0.1, 0.5, and 1.0 mg/L, respectively. The analytical report for concentration verification can be found in the Appendix beginning on page 194. The mean of daily time-weighted average (MIRAN®) concentrations (± standard deviation) for each exposure were 0.11 ± 0.0.1, 0.54 ± 0.03, and 1.09 ± 0.12 mg/L. The mean weekly analytical concentration for the 0.1 mg/L group was 26% higher than the target concentration; the mean weekly analytical concentrations for the 0.5 and 1.0 mg/L groups and the mean daily time-weighted average concentrations for all groups were within 10% of the target concentrations. Nominal concentrations were 0.15 ± 0.01, 0.57 ± 0.03, and 1.36 ± 0.13 mg/L for the same groups. No test substance was detected in the control chamber. Mean chamber temperatures for the 0.0, 0.1, 0.5, and 1.0 mg/L groups were, respectively, 21.0 ± 0.3, 20.8 ± 0.3, 20.6 ± 0.3, and 20.5 ± 0.4°C, and mean chamber relative humidity were, respectively, 66.3 ± 4.6, 66.4 ± 4.2, 66.4 ± 4.1, and 66.9 ± 4.6%. Daily mean values for each exposure are provided in the Appendix.

Mortality

No mortality occurred during the study.

Clinical Observations

Clinical signs observed during exposure are summarized on pages 33 - 34 followed by summaries of clinical examinations prior to and following exposure. Each clinical sign observed during the 6-hour exposure period is listed for each group, as is each clinical sign observed before or after exposure. Individual animal data are presented in the Appendix.

Animals exposed to 1.0 mg/L had reduced activity levels of minimal severity during exposure on Days 7-29. Reduced activity is defined as less movement, decreased alertness, and slower response to tapping on the chamber wall compared with activity levels exhibited by control animals. The 0.1 and 0.5 mg/L animals appeared normal during exposure.

After exposure, porphyrin discharges or dried porphyrin stains were observed around the nose or eyes for one to two 0.5 or 1.0 mg/L male rats on 1 or 2 days, for three 0.1 mg/L female rats on 1-3 days, for all 0.5 mg/L female rats on 1-10 days and for four 1.0 mg/L female rats on 7-9 days. Porphyrin stains were also observed in the morning prior to exposure, though not as frequently for the female rats. All other observations were considered to be incidental to treatment; these observations included inguinal haircoats which were wet or stained with urine, reduced feces, softened feces, red discoloration of the mouth, and dehydration.

Body Weight and Feed Consumption

Mean feed consumption data are presented in graph and tabular form on pages 40 - 41 (males) and 42 - 43 (females). Mean body weights are presented in graph and tabular form on pages 44 - 46 (males) and pages 47 - 49 (females). Individual animal data are presented in the Appendix.

The mean feed consumption was lower ($p \leq 0.05$) for 1.0 mg/L male rats on Day 7 when compared with the control group. The mean feed consumption for male rats from the 0.1 and 0.5 mg/L groups and for female rats from all exposure levels were comparable with the controls throughout the study.

Mean body weights for male and female rats from all exposure levels were comparable with the controls throughout the study.

Hematology

Mean hematology values and analysis of blood cell morphology are presented in summary tables on pages 50 -53. Individual animal data are presented in the Appendix.

For male rats, the mean white blood cell count was lower ($p \leq 0.05$) for the 1.0 mg/L group, and the mean platelet count was lower ($p \leq 0.05$) for the 0.1 mg/L group when compared with the control group. For female rats, mean red blood cell count, hemoglobin concentration, and hematocrit level were higher ($p \leq 0.05$) for the 0.5 mg/L group and mean prothrombin time was higher ($p \leq 0.05$) for the 1.0 mg/L group when compared with the control group. All other hematologic parameters and cell morphology for male and female rats from all exposure levels were comparable with the control groups.

The lower mean platelet count observed for the 0.1 mg/L male group and the higher mean red blood cell count, hemoglobin concentration, and hematocrit level observed for the 0.5 mg/L female group were not considered treatment-related because they were not observed in a concentration dependent manner.

Clinical Chemistry

Mean clinical chemistry values are presented in summary tables on pages 54 (males) and 55 (females). Individual animal data are presented in the Appendix.

For male rats, mean urea nitrogen and sodium levels were higher ($p \leq 0.05$) for the 0.5 and 1.0 mg/L groups, and the mean albumin/globulin ratio was higher ($p \leq 0.05$) for the 1.0 mg/L group when compared with the control group. For female rats, mean urea nitrogen and potassium levels were higher ($p \leq 0.05$) for all exposure levels, and the glucose level was higher ($p \leq 0.05$) for the 0.5 and 1.0 mg/L groups when compared with the control group. All other clinical chemistry parameters for rats from all exposure levels were comparable with the control groups.

Organ Weights

The mean terminal body weights and absolute and relative (to body weight) organ weights are presented in the summary tables on pages 56 - 57 (male) and 58 (female). Individual animal data are presented in the Appendix.

Mean relative (to body weight) heart weights were higher ($p \leq 0.05$) for the 0.5 and 1.0 mg/L male groups and for all exposed female groups when compared with the control group. The mean relative liver weight was higher ($p \leq 0.05$) for the 1.0 mg/L male group, and mean absolute and relative liver weights were higher ($p \leq 0.05$) in a concentration-dependent manner for all exposed female groups when compared with the control group. The mean absolute epididymides weights and mean absolute and relative adrenal gland weights were lower ($p \leq 0.05$) for the 1.0 mg/L male group when compared with the control group. The mean absolute kidney weight was higher ($p \leq 0.05$) for the 0.1 mg/L female group, and the relative kidney weights were higher ($p \leq 0.05$) for all exposed female groups when compared with the control group. The mean relative spleen weight was higher for the 0.1 mg/L female group when compared with the control group; this change was not considered treatment-related because it occurred in the 0.1 mg/L group only. Mean terminal body weights and all other absolute and relative (to body weight) organ weights for male and female rats from all exposure levels were comparable with those of the respective control group.

Gross Pathology

See the Gross Pathology Report beginning on page 59 for details of the necropsy examinations.

For the 1.0 mg/L group, exposure-related changes, observed at the time of necropsy, consisted of minor enlargement of the heart (1/5 male rats), minor to moderate pallor of the heart (1/5 male and 2/5 female rats), and minimal or moderate (4/5 male rats) and minimal to severe (4/5 female rats) pallor of the liver. For the 0.5 mg/L group exposure-related changes consisted of minimal (2/5 male rats) and minor to moderate (2/5 female rats) pallor of the heart, and minimal (1/5 male rats) and minimal to severe (5/5 female rats) pallor of the liver. For the 0.1 mg/L group exposure-related changes consisted of minor to severe pallor of the liver (3/5 female rats). No other exposure-related changes were detected for rats from all exposure levels on necropsy examinations. No other exposure-related changes were observed in any other group. All other lesions were considered incidental to exposure to the test substance.

Histopathology

See the Histopathology Report beginning on page 75 for details of the microscopic examination of tissue.

Histopathologic examination of tissues indicated exposure-related effects in the heart, liver, testes, and epididymides. Lesions in the heart include muscle cell vacuolation, myocarditis, and muscle fiber degeneration. These lesions were observed at all exposure levels for both males and females. Lesions in the liver include hepatocellular cytoplasmic vacuolation which was observed at all exposure levels for both males and females. Lesions in the testes and epididymides consisted of spermatid and/or spermatozoa degeneration which was seen only for the 1.0 mg/L group. Additionally, there was a mild decrease in cellularity within the sternal bone marrow of three 1.0 mg/L female rats and one 0.5 mg/L female rat, as well as a minimal decrease in cellularity in the sternal bone marrow of one 0.1 mg/L female rat. No other exposure-related changes were observed during the histopathology examinations. Slightly greater than expected degrees of cytoplasmic vacuolation of renal tubules were noted for three of five 1.0 mg/L female rats.

DISCUSSION

Animals were exposed to vapor concentrations of 0.0, 0.1, 0.5, or 1.0 mg/L of the test substance for 21 exposures over a 30 day period. Exposure to the test substance produced no compound-related mortality and only minimal signs of overt toxicity. Reduced activity was observed on Day 7 to 29 in animals exposed to 1.0 mg/L, but only during exposure. Therefore, the reduced activity resulting from exposure to the test substance was transient. Post-exposure, porphyrin nasal discharges were observed for the 0.5 and 1.0 mg/L male groups and from all female test substance exposure groups when compared with the control group; the incidence was higher for the female rats when compared with the male rats. These discharges may be indicative of slight irritation of the upper respiratory tract by the vaporized test substance; however, no changes to the nasal passages were observed microscopically which would indicate that the test substance was irritating to the mucous membranes. Other signs of potential toxicity were also transient. Initially, a lower mean feed consumption was observed for the 1.0 mg/L male group; no other changes in feed consumption were observed. No alterations in body weight were observed. Based on clinical observations, body weights, and feed consumption values, signs of overt toxicity were minimal and transient.

When the tissues were examined histopathologically, exposure-related effects were observed in the heart, testes, epididymides, and liver. Heart effects consisted of elevated mean heart weights for the 0.5 and 1.0 mg/L male groups and all exposed female groups. Histopathologically, myocyte vacuolation, myocarditis, and muscle fiber degeneration were observed in the hearts for all test substance exposed male and female groups. These effects were considered by the pathologist to be suggestive of a hypersensitivity myocarditis although there was an absence of eosinophils. However, it is possible that exposure to the test substance did result in changes to the heart that are consistent with the hypersensitivity that is associated with exposure to cyclopropane, an inhalation anesthetic (Cavender, F., 1994; Van Vleet, et. al., 1991). Exposure to high concentrations of cyclopropane can result in elevated levels of catecholamine, but with conflicting reports as to whether the increase was for epinephrine (Deutsch *et al.*, 1962) or norepinephrine (Gardier *et al.*, 1967). Regardless of which catecholamine is elevated, the result is a hypersensitivity to catecholamines which may lead to lesions similar to those observed in this study. Since the test substance and cyclopropane have structural similarities, it seems likely that the test substance may also affect the heart in a similar fashion as cyclopropane. In addition, it is not clear whether the reduced adrenal weight observed for the 1.0 mg/L male group reflects excessive secretion of epinephrine from this gland caused by exposure to the test substance or if this change is coincidental with the primary effect on the heart.

In addition to increases in the level of epinephrine, cyclopropane derivatives such as cyclopropane carboxylate have been reported to decrease fatty acid oxidation in rat myocytes (Bahl *et al.*, 1978), and inhibit glycolysis and gluconeogenesis in skeletal muscle, kidney, and liver (Senior and Sheratt, 1968; Bahl *et al.*, 1978). These biochemical changes may be associated with the observation of pale heart and liver tissue noted at necropsy, and may be related to cytoplasmic vacuolization observed microscopically in the heart and liver. The

biochemical effects on the liver do not appear to be related to increases in serum glucose levels since inhibition of glycolysis and gluconeogenesis would not be expected to increase serum glucose, but could be related to increased BUN levels.

In addition to adverse effects on the heart, testicular and epididymal spermatid and/or spermatozoa degeneration was observed for the 1.0 mg/L male group. The mean absolute, but not relative, epididymides weight was lower for the 1.0 mg/L male group, reflecting the reduced numbers of sperm present. However, there was no change in absolute or relative testes weight, and no microscopic changes in the testes other than degeneration of the spermatozoa for the 1.0 mg/L male group. Other groups were not affected. These findings suggest that a primary effect on the testes is reduction in sperm production, but that the lesions are reversible since spermatogonia were not affected.

Mean relative kidney weights were higher for all exposed female groups and the mean absolute kidney weight was higher for the 0.1 mg/L female group when compared with the control group. Histopathologically, mild to moderate cytoplasmic vacuolation was observed in the proximal convoluted tubules of the kidney for three of the 1.0 mg/L female rats versus minimal cytoplasmic vacuolation observed for the control female rats. This slightly greater than expected degree of cytoplasmic vacuolation was considered by the pathologist to represent either a cytologic alteration reflective of postmortem autolytic changes, or the normal range of variability for this strain of rat.

Decreased cellularity within the sternal bone marrow was observed for one 0.1 mg/L, one 0.5 mg/L, and three 1.0 mg/L female rats. While the degree of marrow cellularity may possibly still be within a normal range, slightly lower numbers of erythropoietic and myelopoietic cells were observed when compared with the control female rats. Thus, the toxicological significance of this change is not clear.

Additionally, a number of changes in hematology were observed which may be treatment-related. However, the biological significance of these changes is unclear. A lower mean white blood cell count observed for the male 1.0 mg/L group, while possibly clinically significant, was not considered toxicologically significant, and the slightly higher mean prothrombin time observed for the female 1.0 mg/L group was not considered toxicologically significant. The very slightly higher (1%) mean sodium levels observed for the 0.5 and 1.0 mg/L male groups and the slightly higher mean albumin/globulin ratio observed for the 1.0 mg/L male group were not considered biologically significant.

CONCLUSION

Based on the heart lesions which were observed at 0.1 mg/L, the lowest concentration tested, a no-observed-effect concentration (NOEC) was not determined. In addition, the effect on the heart was considered to be adverse. Thus, a no-observed-adverse-effect concentration (NOAEC) was not identified. Effects on the liver which were considered adaptive, and were not considered to constitute an adverse effect.

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Summary of Exposure Conditions

TARGET CONCENTRATION (mg/L)		0.0	0.1	0.5	1.0
NUMBER OF EXPOSURES		21	21	21	21
WEEKLY MEAN CONCENTRATION (mg/L)	Mean	0.000	0.126	0.546	0.970
Measured on Days 1, 8, 15, 21, and 29	SD	0.000	0.015	0.019	0.100
	n	5	5	5	5
MEAN DAILY TIME WEIGHTED AVERAGE	Mean	0.00	0.11	0.54	1.09
CONCENTRATION (mg/L)	SD	0.00	0.01	0.03	0.12
	n	21	21	21	21
Extremes of Daily Values	Low	0.00	0.10	0.52	0.94
	High	0.00	0.13	0.66	1.55
NOMINAL CONCENTRATION (mg/L)	Mean	0.00	0.15	0.57	1.36
	SD	0.00	0.01	0.03	0.13
	n	21	21	21	21
Extremes of Daily Values	Low	0.00	0.13	0.54	1.13
	High	0.00	0.16	0.66	1.65
TEMPERATURE (°C)	Mean	21.0	20.8	20.6	20.5
	SD	0.3	0.3	0.3	0.4
	n	252	252	252	252
RELATIVE HUMIDITY (%)	Mean	66.3	66.4	66.4	66.9
	SD	4.6	4.2	4.1	4.6
	n	252	252	252	252
AIRFLOW (Lpm)	Mean	131.8	149.6	147.8	137.4
	SD	6.8	8.8	6.9	5.2
	n	21	21	21	21

Summary of During Exposure Clinical Signs - Male Rats

		Day of Onset	Last Day of Observation	Number of Rats Displaying Sign	Mean of Daily Maximum Severity	Frequency (Days)
0.0 mg/L	Normal	0	29	5	-	21
0.1 mg/L	Normal	0	29	5	-	21
0.5 mg/L	Normal	0	29	5	-	21
1.0 mg/L	Normal	0	29	5	--	21
	Reduced Activity	7	29	5	1.00	16

The animals were observed hourly during exposure. All the clinical signs observed are listed on this summary even if the clinical signs were observed for a short period of time during exposure.

Severities: 1 = Minimal, 2 = Minor, 3 = Moderate, 4 = Severe

Summary of During Exposure Clinical Signs - Female Rats

		Day of Onset	Last Day of Observation	Number of Rats Displaying Sign	Mean of Daily Maximum Severity	Frequency (Days)
0.0 mg/L	Normal	0	29	5	-	21
0.1 mg/L	Normal	0	29	5	-	21
0.5 mg/L	Normal	0	29	5	-	21
1.0 mg/L	Normal	0	29	5	-	21
	Reduced Activity	7	29	5	1.00	16

The animals were observed hourly during exposure. All the clinical signs observed are listed on this summary even if the clinical signs were observed for a short period of time during exposure.

Severities: 1 = Minimal, 2 = Minor, 3 = Moderate, 4 = Severe

GROUPED SUMMARY OF CLINICAL SIGNS - MALE RATS

OBSERVATION PERIOD - A.M.

CLINICAL SIGN	NO. OF ANIMALS	MEAN ONSET DAY	ONSET S.D. #DAYS	ANIMALS AFFECTED
GROUP 1 - 0.000 MG/L				
* NORMAL	5			501-505
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	501-505
GROUP 2 - 0.100 MG/L				
* NORMAL	5			506-510
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	506-510
GROUP 3 - 0.500 MG/L				
* NORMAL	4			511-513,515
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	511-515
NOSE				
DRIED PORPHYRIN DISCHARGE	1	30	0	514
GROUP 4 - 1.000 MG/L				
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	516-520
FECES				
DECREASED VOLUME	5	1	0	516-520
SOFTENED	2	5	5	517-518
NOSE				
DRIED PORPHYRIN DISCHARGE	2	30	0	517,520

KEY: *- INDICATES ANIMALS SHOWING ONLY CLINICAL EXAM NORMAL, MORIBUNDITY/MORTALITY CHECK NORMAL, OR CAGESIDE OBSERVATION NORMAL

GROUPED SUMMARY OF CLINICAL SIGNS - MALE RATS

OBSERVATION PERIOD - P.M.

CLINICAL SIGN	NO. OF ANIMALS	MEAN ONSET DAY	ONSET S.D. #DAYS	ANIMALS AFFECTED
GROUP 1 - 0.000 MG/L				
* NORMAL	5			501-505
GROUP 2 - 0.100 MG/L				
* NORMAL	5			506-510
GROUP 3 - 0.500 MG/L				
* NORMAL	3			512-513,515
EYES				
PORPHYRIN TEARS	1	8	0	511
HAIR OF FACE				
DRIED PORPHYRIN DISCHARGE	1	22	0	514
GROUP 4 - 1.000 MG/L				
* NORMAL	4			516-518,520
NOSE				
DRIED PORPHYRIN DISCHARGE	1	10	0	519

KEY: *- INDICATES ANIMALS SHOWING ONLY CLINICAL EXAM NORMAL, MORIBUNDITY/MORTALITY CHECK NORMAL, OR CAGESIDE OBSERVATION NORMAL

GROUPED SUMMARY OF CLINICAL SIGNS - FEMALE RATS

OBSERVATION PERIOD - A.M.

CLINICAL SIGN	NO. OF ANIMALS	MEAN ONSET DAY	ONSET S.D. #DAYS	ANIMALS AFFECTED
GROUP 1 - 0.000 MG/L				
* NORMAL	4			522-525
FEED SPILLAGE	1	18	0	524
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	521-525
HAIR OF INGUINAL REGION				
HAIRCOAT, WET BY URINE	1	1	0	521
HAIRCOAT, DRY URINE STAIN	1	2	0	521
GROUP 2 - 0.100 MG/L				
* NORMAL	4			526-528,530
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	526-530
NOSE				
PORPHYRIN NASAL DISCHARGE	1	1	0	529
HAIR OF INGUINAL REGION				
HAIRCOAT, DRY URINE STAIN	1	29	0	529
GROUP 3 - 0.500 MG/L				
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	531-535
FECES				
DECREASED VOLUME	4	5	7	532-535
NOSE				
DRIED PORPHYRIN DISCHARGE	3	16	14	532,534-535
HAIR OF INGUINAL REGION				
HAIRCOAT, DRY URINE STAIN	2	24	8	532,535
HAIRCOAT, WET BY URINE	1	30	0	531
HAIR OF FACE				
DRIED PORPHYRIN DISCHARGE	1	30	0	535

KEY: *- INDICATES ANIMALS SHOWING ONLY CLINICAL EXAM NORMAL, MORIBUNDITY/MORTALITY CHECK NORMAL, OR CAGESIDE OBSERVATION NORMAL

GROUPED SUMMARY OF CLINICAL SIGNS - FEMALE RATS

OBSERVATION PERIOD - A.M.

CLINICAL SIGN	NO. OF ANIMALS	MEAN ONSET DAY	ONSET S.D. #DAYS	ANIMALS AFFECTED
GROUP 4 - 1.000 MG/L				
DEHYDRATION	1	2	0	538
INDUCED DEATH,METOFANE,EXSANGUINATION	5	30	0	536-540
FECES				
DECREASED VOLUME	5	1	0	536-540
HAIR OF FACE				
DISCOLORATION,RED	1	3	0	538
DRIED PORPHYRIN DISCHARGE	4	30	0	536-539
NOSE				
DRIED PORPHYRIN DISCHARGE	1	6	0	539
HAIR OF INGUINAL REGION				
HAIRCOAT, WET BY URINE	2	30	0	536-537

KEY: *- INDICATES ANIMALS SHOWING ONLY CLINICAL EXAM NORMAL, MORIBUNDITY/MORTALITY CHECK NORMAL, OR CAGESIDE OBSERVATION NORMAL

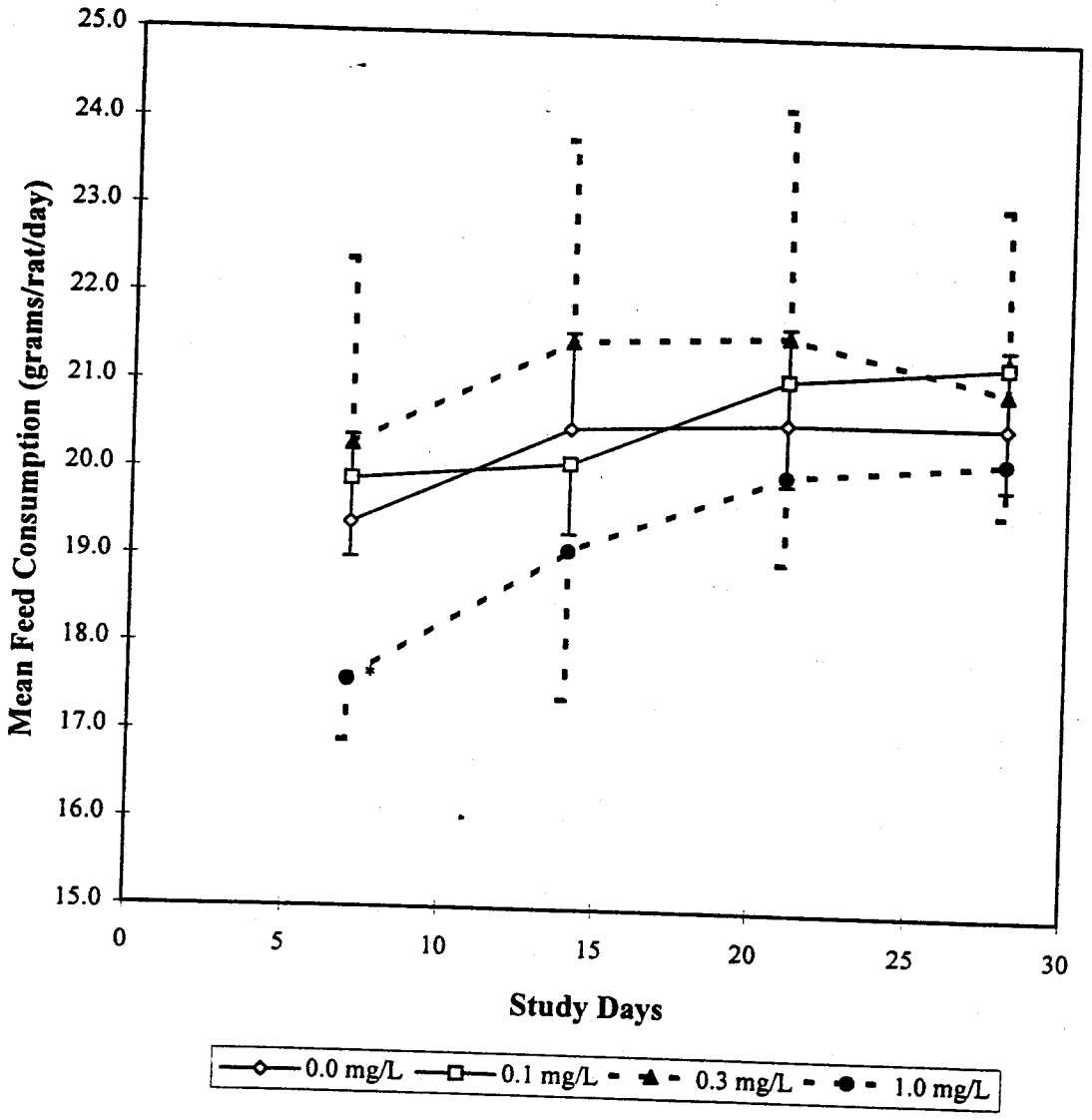
GROUPED SUMMARY OF CLINICAL SIGNS - FEMALE RATS

OBSERVATION PERIOD - P.M.

CLINICAL SIGN	NO. OF ANIMALS	MEAN ONSET DAY	ONSET S.D. #DAYS	ANIMALS AFFECTED
GROUP 1 - 0.000 MG/L				
* NORMAL	4			522-525
HAIR OF INGUINAL REGION				
HAIRCOAT, DRY URINE STAIN	1	1	0	521
HAIRCOAT, WET BY URINE	1	22	0	521
GROUP 2 - 0.100 MG/L				
* NORMAL	2			527-528
NOSE				
DRIED PORPHYRIN DISCHARGE	1	15	0	530
HAIR OF INGUINAL REGION				
HAIRCOAT, WET BY URINE	1	17	0	529
HAIR OF FACE				
DRIED PORPHYRIN DISCHARGE	2	22	1	526,529
GROUP 3 - 0.500 MG/L				
NOSE				
DRIED PORPHYRIN DISCHARGE	5	14	7	531-535
MOUTH				
DISCOLORATION,RED	1	10	0	532
HAIR OF INGUINAL REGION				
HAIRCOAT, WET BY URINE	1	15	0	535
HAIRCOAT, DRY URINE STAIN	2	21	0	532,535
HAIR OF FACE				
DRIED PORPHYRIN DISCHARGE	3	23	2	531-532,535
GROUP 4 - 1.000 MG/L				
* NORMAL	1			540
DEHYDRATION	1	2	0	538
HAIR OF FACE				
DISCOLORATION,RED	1	2	0	538
DRIED PORPHYRIN DISCHARGE	4	22	1	536-539
NOSE				
DRIED PORPHYRIN DISCHARGE	4	15	1	536-539
EYES				
PORPHYRIN TEARS	1	22	0	537
HAIR OF INGUINAL REGION				
HAIRCOAT, WET BY URINE	1	29	0	536

KEY: *- INDICATES ANIMALS SHOWING ONLY CLINICAL EXAM NORMAL, MORIBUNDITY/MORTALITY CHECK NORMAL, OR CAGESIDE OBSERVATION NORMAL

Mean Feed Consumption - Male Rats

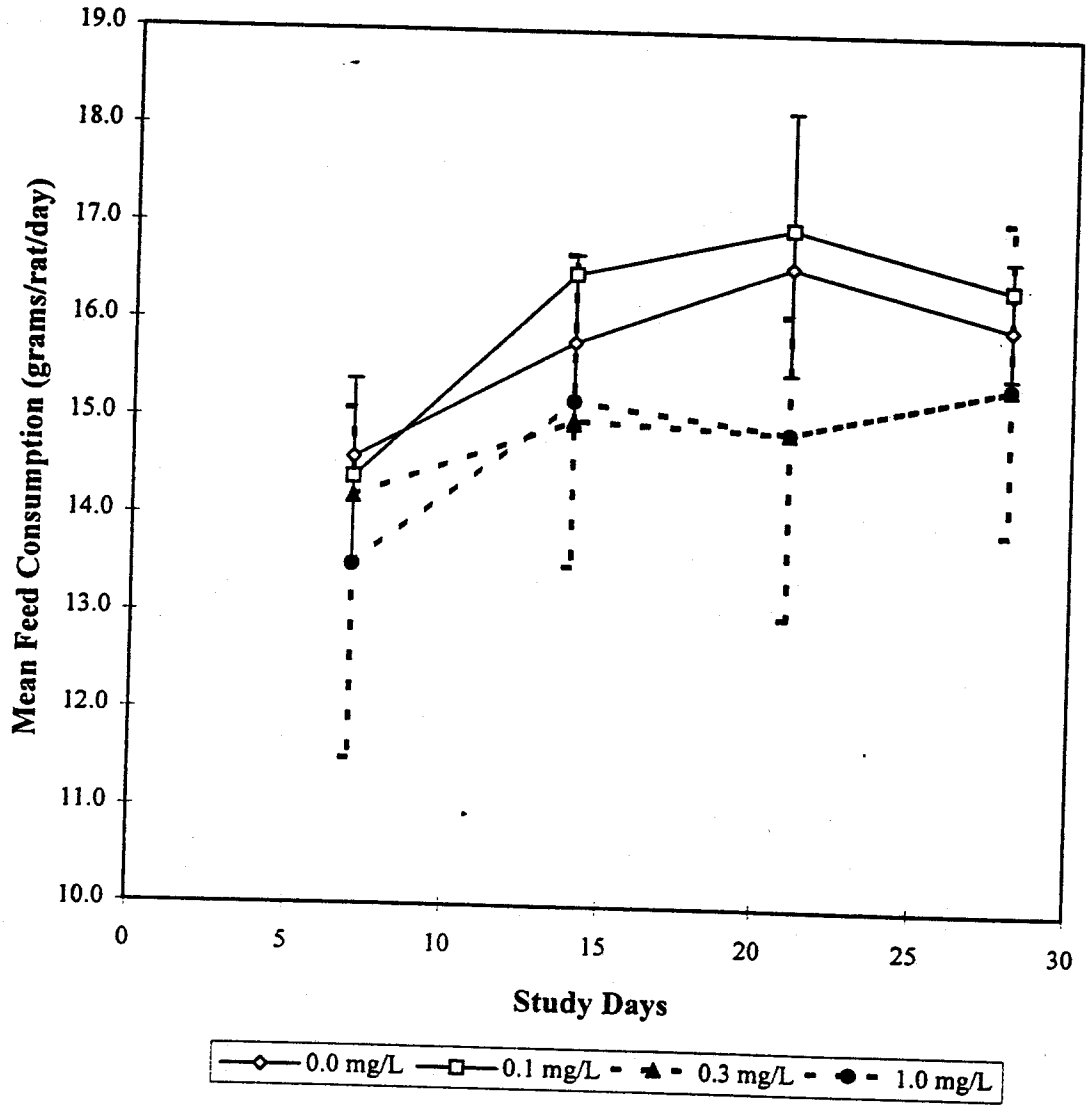


MEAN FOR FEED CONSUMPTION (GRAMS/ANIMAL/DAY) - MALE RATS

	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
WEEK # 1				
DAY 7	19.4 1.0 5	19.9 0.9 5	20.3 2.1 5	17.6 * 0.7 5
WEEK # 2				
DAY 14	20.5 1.1 5	20.1 0.8 5	21.5 2.3 5	19.1 1.7 5
WEEK # 3				
DAY 21	20.6 1.1 5	21.1 1.2 5	21.6 2.6 5	20.0 1.0 5
WEEK # 4				
DAY 28	20.6 0.9 5	21.3 1.4 5	21.0 2.1 5	20.2 0.6 5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, +/- STANDARD DEVIATION, AND NUMBER PER GROUP
* - STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

Mean Feed Consumption - Female Rats

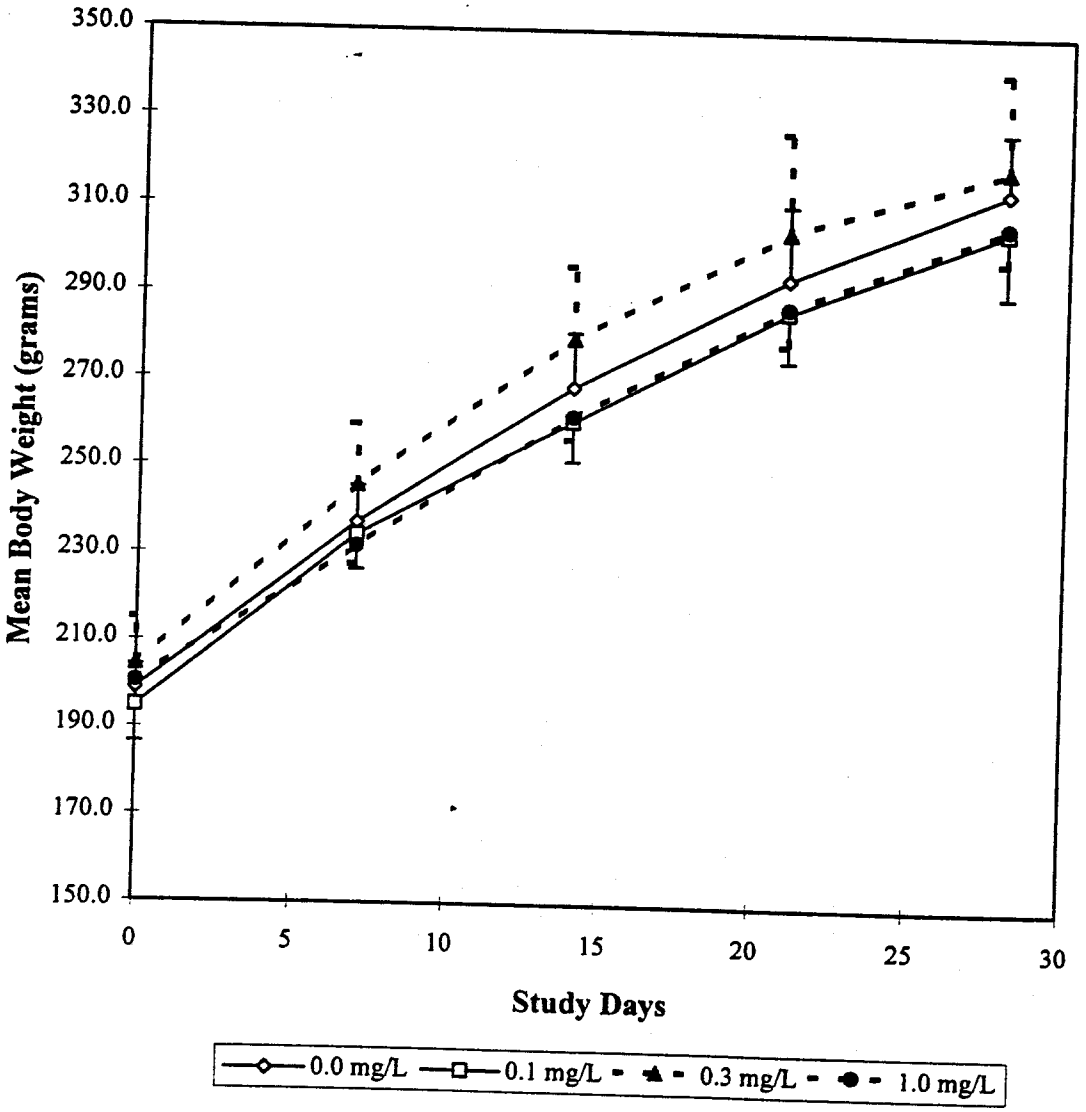


MEAN FEED CONSUMPTION (GRAMS/ANIMAL/DAY) - FEMALE RATS

	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
WEEK # 1				
DAY 7	14.6 0.8 5	14.4 0.9 5	14.2 0.9 5	13.5 2.0 5
WEEK # 2				
DAY 14	15.8 0.9 5	16.5 1.6 5	15.0 1.7 5	15.2 1.7 5
WEEK # 3				
DAY 21	16.6 1.6 5	17.0 1.5 5	14.9 1.2 5	14.9 1.9 5
WEEK # 4				
DAY 28	16.0 0.7 5	16.4 0.9 5	15.4 1.7 5	15.4 1.5 5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION, AND NUMBER PER GROUP

Mean Body Weight - Male Rats



MEAN FOR BODY WEIGHT (GRAMS) - MALE RATS

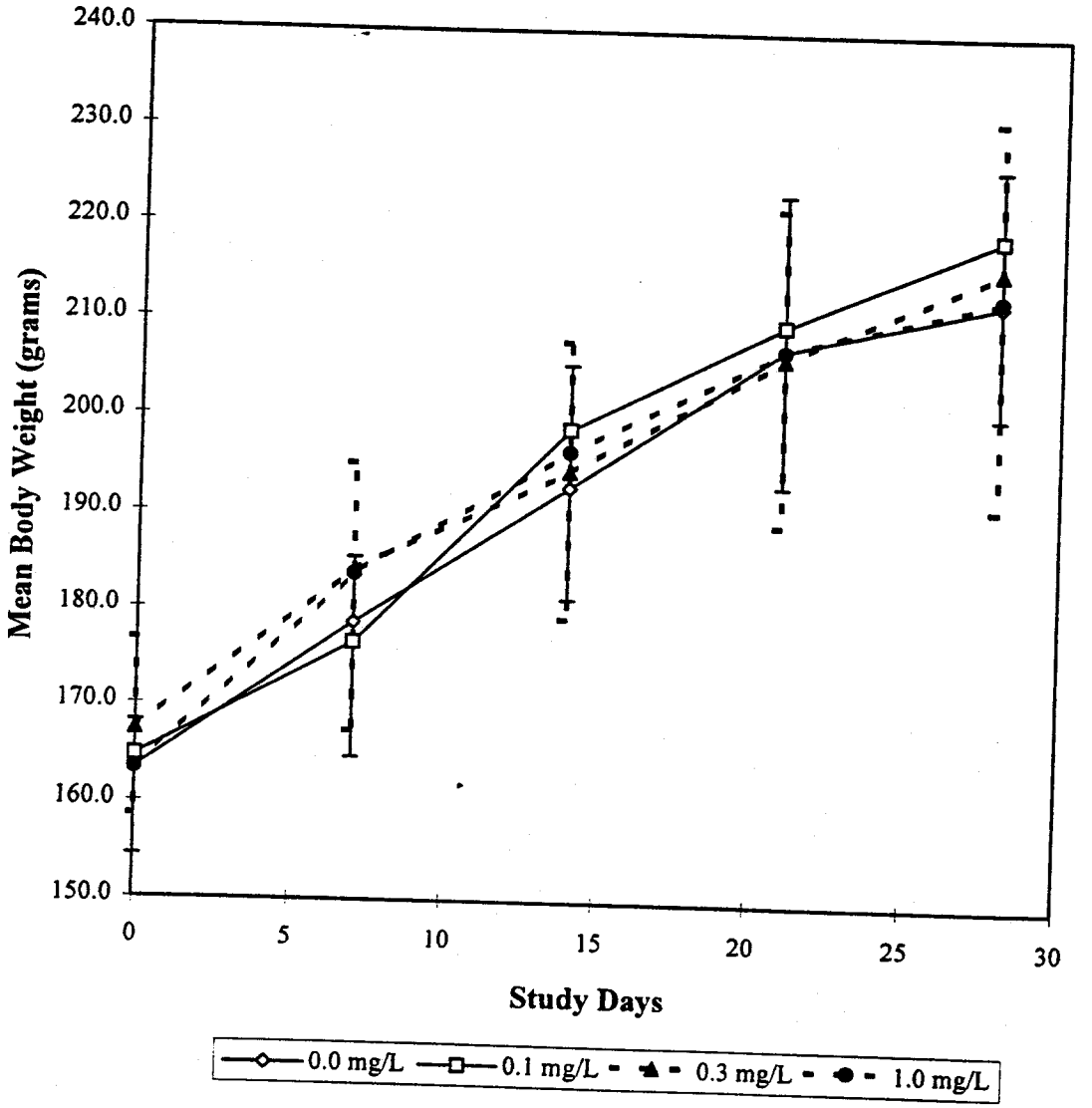
	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
WEEK # 1				
DAY 0	198.9 5.3 5	194.9 8.3 5	204.5 10.5 5	200.5 5.3 5
DAY 7	237.3 8.3 5	234.5 7.9 5	245.8 13.9 5	231.9 4.1 5
WEEK # 2				
DAY 14	268.6 12.5 5	260.6 9.1 5	279.8 16.5 5	261.8 5.3 5
WEEK # 3				
DAY 21	294.0 16.5 5	286.4 11.3 5	304.6 22.6 5	287.4 8.5 5
WEEK # 4				
DAY 28	314.3 13.6 5	305.4 14.6 5	319.4 22.1 5	306.5 8.0 5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, SANDARD DEVIATION, AND NUMBER PER GROUP

Mean Body Weight Change (Grams) - Male Rats

	0.0 m/L	0.1 m/L	0.3 m/L	1.0 m/L
Days 0-7	34.8 9.9 5	39.7 4.5 5	41.4 6.3 5	31.4 5.2 5
Days 7-14	34.9 11.3 5	26.1 8.3 5	33.9 4.9 5	29.9 5.6 5
Days 14-21	25.4 4.7 5	25.9 6.1 5	24.8 6.8 5	25.6 4.8 5
Days 21-28	20.3 5.5 5	19.0 4.4 5	14.8 2.4 5	19.1 1.6 5

Mean Body Weight - Female Rats



Archive Form

MEAN FOR BODY WEIGHT (GRAMS) - FEMALE RATS

KAN: 9[illegible] 0.0 MG/L 0.1 MG/L 0.5 MG/L 1.0 MG/L

HAEL# No: _____

DAY 0	163.4	164.7	167.4	163.4
	4.8	10.2	9.3	4.8
	5	5	5	5
DAY 7	178.6	176.5	184.1	183.7
	6.8	11.8	11.0	16.3
	5	5	5	5
DAY 14	192.8	198.8	194.5	196.5
	12.6	19.6	13.3	17.3
	5	5	5	5
DAY 21	207.3	209.8	206.4	207.2
	16.0	16.6	15.4	18.0
	5	5	5	5
DAY 28	212.3	219.1	215.7	212.8
	14.0	18.5	15.5	21.6
	5	5	5	5

Laboratory: Eastman Chemical Company

WEEK # 2

Study Type: Evaluation of Oncorhynchus mykiss (rainbow trout) [illegible]

WEEK # 3

Materials Archived:

X Final Report

___ Raw Data

WEEK # 4

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION, AND NUMBER PER GROUP

Archived by: Jennifer Fiel Date: 5-13-98

Received by: Judy T. Farrell Date: 5-13-98

Mean Body Weight Change - Female Rats

	0.0 m/L	0.1 m/L	0.3 mg/L	1.0 mg/L
Days 0-7	15.2	11.8	16.7	20.3
	2.3	6.6	5.9	12.2
	5	5	5	5
Days 7-14	14.2	22.3	10.3	12.8
	7.2	8.9	12.4	6.7
	5	5	5	5
Days 14-21	14.5	11.0	11.9	10.6
	6.8	4.8	8.9	11.3
	5	5	5	5
Days 21-28	5.0	9.3	9.3	5.6
	6.2	2.7	1.5	5.1
	5	5	5	5

SUMMARY HEMATOLOGY DETERMINATION - MALE RATS

ANALYTICAL MATERIAL: BLOOD
SAMPLE DAY # 30

	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
WBC/MM3 X3	9.1	11.5	7.2	4.9 *
	2.1	2.4	2.0	2.5
	5	4	5	4
RBC/MM3 X6	8.2	8.5	8.5	8.4
	0.6	0.2	0.4	0.4
	5	4	5	4
HB CONC , G/DL	15.7	16.1	16.2	16.1
	0.6	0.2	0.8	0.7
	5	4	5	4
HCT , %	45.6	47.2	46.5	46.6
	1.7	0.8	1.6	1.5
	5	4	5	4
MCV , U3	56.0	55.6	54.5	55.4
	2.3	2.1	2.1	1.1
	5	4	5	4
MCH , UUG	19.3	18.9	18.9	19.1
	0.7	0.6	0.5	0.5
	5	4	5	4
MCHC , %	34.4	34.1	34.8	34.5
	0.3	0.7	1.1	0.6
	5	4	5	4
PLATELETS/MM3 X3	971.6	767.8 *	976.4	914.0
	113.3	25.3	164.5	28.9
	5	4	5	4
POLYS , %	11.6	7.8	22.8	15.6
	7.8	3.3	11.6	8.1
	5	5	5	5
BANDS , %	0.2	0.2	1.2	0.0
	0.4	0.4	2.7	0.0
	5	5	5	5
LYMPHOCYTES,%	83.0	88.6	71.2	78.8
	9.8	2.4	13.8	9.7
	5	5	5	5
MONOCYTES,%	4.0	2.2	4.2	4.8
	3.3	1.1	1.5	3.0
	5	5	5	5
EOSINOPHIL,%	1.2	1.0	0.2	0.4
	1.6	0.7	0.4	0.5
	5	5	5	5
BASOPHIL,%	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0
	5	5	5	5
LYMPHOCYTES ATYPICAL, %	0.0	0.2	0.4	0.4
	0.0	0.4	0.5	0.5
	5	5	5	5
NUCLEATED RBC/100WBC	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0
	5	5	5	5
PROTHROMBIN TIME,SEC	16.6	15.9	16.7	17.8
	1.3	0.8	1.3	3.6
	5	5	5	5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION, AND NUMBER PER GROUP
* -STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

SUMMARY HEMATOLOGY DETERMINATION - FEMALE RATS

ANALYTICAL MATERIAL: BLOOD
SAMPLE DAY # 30

	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
WBC/MM3 X3	8.6	5.9	5.9	5.6
	4.0	1.4	1.5	1.4
	3	2	5	4
RBC/MM3 X6	8.0	8.4	8.6 *	8.0
	0.6	0.2	0.2	0.2
	3	2	5	4
HB CONC , G/DL	15.1	15.9	16.0 *	14.7
	0.7	0.5	0.4	0.3
	3	2	5	4
HCT , %	43.3	46.2	47.2 *	42.9
	2.7	1.6	1.2	0.8
	3	2	5	4
MCV , U3	54.0	54.7	54.6	53.7
	0.7	0.8	0.7	1.0
	3	2	5	4
MCH , UUG	18.8	18.8	18.5	18.4
	0.6	0.1	0.3	0.4
	3	2	5	4
MCHC , %	34.9	34.4	34.0	34.3
	0.8	0.1	0.4	1.0
	3	2	5	4
PLATELETS/MM3 X3	826.7	910.0	932.2	971.8
	73.4	124.5	84.2	133.4
	3	2	5	4
POLYS , %	7.0	9.0	7.6	7.4
	5.4	4.5	2.9	1.1
	5	4	5	5
BANDS , %	0.2	0.3	0.0	0.0
	0.4	0.5	0.0	0.0
	5	4	5	5
LYMPHOCYTES,%	88.4	84.5	86.0	89.8
	7.1	7.3	6.6	2.4
	5	4	5	5
MONOCYTES,%	3.2	4.8	4.4	2.4
	1.6	3.6	3.8	1.1
	5	4	5	5
EOSINOPHIL,%	1.0	1.3	1.6	0.2
	0.0	1.3	2.6	0.4
	5	4	5	5
BASOPHIL,%	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0
	5	4	5	5
LYMPHOCYTES ATYPICAL, %	0.2	0.3	0.4	0.2
	0.4	0.5	0.9	0.4
	5	4	5	5
NUCLEATED RBC/100WBC	0.0	0.0	0.0	0.0
	0.0	0.0	0.0	0.0
	5	4	5	5
PROTHROMBIN TIME,SEC	16.8		17.8	19.6 *
	0.2		0.9	2.1
	4		5	4

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION, AND NUMBER PER GROUP
* -STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

STUDY SUMMARY OF CELL MORPHOLOGY - MALE RATS

ANALYTICAL MATERIAL : BLOOD CELL MORPHOLOGY

DAY OF SAMPLE : 30

GROUP	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
BLOOD ERYTHROCYTES	5	5	5	5
BLOOD LEUKOCYTES	5	5	5	5
BLOOD PLATELETS	5	5	5	5

NUMBERS REPRESENT NUMBER OF ANIMALS EXAMINED, OR IN THE CASE OF ABNORMAL FINDINGS, THE NUMBER OF ANIMALS WITH EACH ABNORMALITY

STUDY SUMMARY OF CELL MORPHOLOGY - FEMALE RATS

ANALYTICAL MATERIAL : BLOOD CELL MORPHOLOGY

DAY OF SAMPLE : 30

GROUP	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
BLOOD ERYTHROCYTES	5	4	5	5
BLOOD LEUKOCYTES	5	4	5	5
BLOOD PLATELETS	5	4	5	5

NUMBERS REPRESENT NUMBER OF ANIMALS EXAMINED, OR IN THE CASE OF ABNORMAL FINDINGS, THE NUMBER OF ANIMALS WITH EACH ABNORMALITY

SUMMARY CLINICAL CHEMISTRY DETERMINATION - MALE RATS

ANALYTICAL MATERIAL: SERUM
SAMPLE DAY # 30

	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
ALT (GPT) , U/L	28.36	27.73	39.53	34.55
	4.01	6.22	9.01	7.63
	5	5	5	5
UREA NITROGEN,MG/DL	15.55	15.49	22.43 *	23.95 *
	1.68	2.10	0.88	3.91
	5	5	5	5
GLUCOSE,MG/DL	94.99	106.31	157.44	130.93
	7.50	10.81	66.18	21.87
	5	5	5	5
CREATININE,MG/DL	0.55	0.53	0.57	0.57
	0.02	0.02	0.02	0.02
	5	5	5	5
SDH , U/L	5.22	6.01	8.49	9.68
	1.18	1.29	2.21	5.42
	5	5	5	5
BILIRUBIN TOTAL,MG/DL	0.06	0.08	0.09	0.10
	0.04	0.05	0.06	0.09
	5	5	5	5
TOTAL PROTEIN,G/DL	5.47	5.27	5.27	5.14
	0.24	0.33	0.42	0.37
	5	5	5	5
A/G RATIO	1.03	1.16	1.11	1.29 *
	0.13	0.11	0.11	0.16
	5	5	5	5
ALBUMIN,G/DL	2.77	2.82	2.76	2.88
	0.11	0.12	0.10	0.15
	5	5	5	5
CHOLESTEROL,MG/DL	44.31	50.26	38.67	37.67
	10.00	5.87	10.28	15.33
	5	5	5	5
TRIGLYCERIDES,MG/DL	22.97	28.50	30.80	35.11
	7.48	7.54	14.24	17.16
	5	5	5	5
CALCIUM , MG/DL	9.99	10.53	10.34	10.07
	0.63	0.48	0.60	0.75
	5	5	5	5
PHOSPHORUS , MG/DL	7.63	8.38	8.39	8.28
	0.75	0.27	0.77	0.32
	5	5	5	5
SODIUM , MEQ/L	144.60	144.40	146.40 *	146.20 *
	0.54	0.89	0.55	0.84
	5	5	5	5
POTASSIUM , MEQ/L	4.63	4.76	5.12	4.86
	0.32	0.29	0.31	0.32
	5	5	5	5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION AND NUMBER PER GROUP
* -STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

SUMMARY CLINICAL CHEMISTRY DETERMINATION - FEMALE RATS

ANALYTICAL MATERIAL: SERUM
SAMPLE DAY # 30

	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
ALT (GPT) , U/L	24.87	29.54	24.54	27.15
	7.60	7.59	5.81	2.08
	5	2	5	5
UREA NITROGEN,MG/DL	20.85	30.97 *	29.47 *	27.69 *
	3.30	2.58	3.96	2.57
	5	2	5	5
GLUCOSE,MG/DL	97.28	104.56	111.18 *	110.45 *
	4.98	2.69	3.82	10.02
	5	2	5	5
CREATININE,MG/DL	0.66	0.63	0.63	0.63
	0.12	0.01	0.09	0.04
	5	2	5	5
SDH , U/L	6.98	9.94	7.34	8.84
	1.78	5.05	1.80	5.11
	5	2	5	5
BILIRUBIN TOTAL,MG/DL	0.11	0.07	0.12	0.10
	0.06	0.04	0.08	0.03
	5	2	5	5
TOTAL PROTEIN,G/DL	5.34	5.30	5.25	5.19
	0.20	0.38	0.26	0.39
	5	2	5	5
A/G RATIO	1.18	1.33	1.36	1.34
	0.15	0.08	0.10	0.10
	5	2	5	5
ALBUMIN,G/DL	2.88	3.02	3.02	2.96
	0.15	0.30	0.21	0.20
	5	2	5	5
CHOLESTEROL,MG/DL	48.76	32.15	41.93	37.11
	6.77	31.61	16.44	17.86
	5	2	5	5
TRIGLYCERIDES,MG/DL	26.60	41.85	61.03	58.48
	6.36	5.16	32.31	40.34
	5	2	5	5
CALCIUM , MG/DL	9.76	9.91	10.08	9.23
	0.42	0.25	0.64	0.32
	5	2	5	5
PHOSPHORUS , MG/DL	7.95	9.05	8.95	8.42
	0.89	1.00	0.77	0.74
	5	2	5	5
SODIUM , MEQ/L	142.40	142.50	141.40	142.00
	1.52	2.12	1.52	1.41
	5	2	5	4
POTASSIUM , MEQ/L	4.31	5.24 *	5.19 *	5.56 *
	0.16	0.35	0.10	0.43
	5	2	5	4

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION AND NUMBER PER GROUP
* -STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

MEAN TERMINAL ORGAN WEIGHTS AND BODY WEIGHTS - MALE RATS

TERMINATION DAY - 30

GROUP		0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
BODY WEIGHT	(G)	271.4	261.9	271.5	258.3
		13.9	13.0	20.1	10.3
		5	5	5	5
HEART	(G)	1.0003	1.0132	1.1307	1.1153
		0.0907	0.0487	0.1406	0.0475
		5	5	5	5
	%	0.3686	0.3876	0.4167 *	0.4320 *
		0.0270	0.0257	0.0454	0.0163
		5	5	5	5
LUNGS	(G)	1.2962	1.2490	1.4773	1.3818
		0.1024	0.1023	0.2681	0.0738
		5	5	5	5
	%	0.4772	0.4776	0.5437	0.5352
		0.0151	0.0422	0.0875	0.0254
		5	5	5	5
LIVER	(G)	7.8293	7.2619	8.5055	8.5882
		0.6880	0.5550	1.2400	0.4723
		5	5	5	5
	%	2.8817	2.7740	3.1224	3.3261 *
		0.1306	0.1779	0.2509	0.1611
		5	5	5	5
KIDNEYS	(G)	2.0850	2.0291	2.0414	1.9541
		0.2273	0.1467	0.2350	0.1479
		5	5	5	5
	%	0.7667	0.7754	0.7503	0.7583
		0.0456	0.0517	0.0309	0.0738
		5	5	5	5
ADRENALS	(G)	0.0554	0.0591	0.0532	0.0406 *
		0.0052	0.0057	0.0067	0.0061
		5	5	5	5
	%	0.0205	0.0226	0.0197	0.0157 *
		0.0026	0.0028	0.0025	0.0022
		5	5	5	5
THYMUS	(G)	0.4498	0.5424	0.4307	0.3431
		0.0686	0.1100	0.0808	0.1110
		5	5	5	5
	%	0.1656	0.2071	0.1592	0.1322
		0.0233	0.0396	0.0301	0.0397
		5	5	5	5
SPLEEN	(G)	0.6519	0.6700	0.5620	0.5094
		0.1199	0.1202	0.0990	0.0620
		5	5	5	5
	%	0.2399	0.2570	0.2063	0.1972
		0.0386	0.0509	0.0286	0.0221
		5	5	5	5
BRAIN	(G)	1.8624	1.8207	1.8291	1.7649
		0.0797	0.0387	0.1563	0.0654
		5	5	5	5
	%	0.6868	0.6963	0.6757	0.6846
		0.0227	0.0283	0.0644	0.0454
		5	5	5	5
TESTES	(G)	3.2486	3.2033	3.0119	2.7974
		0.2639	0.2098	0.3576	0.3285
		5	5	5	5
	%	1.1958	1.2242	1.1090	1.0853
		0.0401	0.0746	0.0965	0.1400
		5	5	5	5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION, AND NUMBER PER GROUP
* - STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

MEAN TERMINAL ORGAN WEIGHTS AND BODY WEIGHTS - MALE RATS

TERMINATION DAY - 30

GROUP		0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
BODY WEIGHT	(G)	271.4	261.9	271.5	258.3
		13.9	13.0	20.1	10.3
		5	5	5	5
EPIDIDYMIDES	(G)	0.9400	0.8692	0.8868	0.7597 *
		0.0662	0.0983	0.0514	0.0782
		5	5	5	5
	%	0.3478	0.3326	0.3272	0.2947
		0.0385	0.0400	0.0141	0.0346
		5	5	5	5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION, AND NUMBER PER GROUP
* - STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

MEAN TERMINAL ORGAN WEIGHTS AND BODY WEIGHTS - FEMALE RATS

TERMINATION DAY - 30

GROUP		0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
BODY WEIGHT	(G)	178.6	180.0	179.0	176.0
		12.8	12.3	14.3	16.4
		5	4	5	5
HEART	(G)	0.7217	0.8715	0.7943	0.8072
		0.0886	0.1011	0.1049	0.1058
		5	5	5	5
	%	0.4029	0.4636 *	0.4436 *	0.4575 *
		0.0214	0.0164	0.0442	0.0241
		5	4	5	5
LUNGS	(G)	1.0444	1.1207	1.0246	0.9978
		0.0381	0.1450	0.0824	0.0801
		5	5	5	5
	%	0.5863	0.5905	0.5736	0.5681
		0.0295	0.0195	0.0430	0.0348
		5	4	5	5
LIVER	(G)	4.8871	6.3785 *	6.8868 *	6.9077 *
		0.2310	0.7711	0.6650	0.5962
		5	5	5	5
	%	2.7436	3.3795 *	3.8499 *	3.9471 *
		0.1644	0.2495	0.2750	0.4532
		5	4	5	5
KIDNEYS	(G)	1.2709	1.4896 *	1.4070	1.4023
		0.1189	0.1042	0.1323	0.0740
		5	5	5	5
	%	0.7118	0.8143 *	0.7855 *	0.7997 *
		0.0443	0.0363	0.0275	0.0496
		5	4	5	5
ADRENALS	(G)	0.0584	0.0656	0.0536	0.0502
		0.0072	0.0105	0.0074	0.0105
		5	5	5	5
	%	0.0328	0.0347	0.0300	0.0284
		0.0042	0.0047	0.0041	0.0041
		5	4	5	5
THYMUS	(G)	0.2675	0.2965	0.2412	0.2301
		0.0504	0.0577	0.1061	0.0545
		5	5	5	5
	%	0.1499	0.1668	0.1317	0.1296
		0.0268	0.0419	0.0508	0.0224
		5	4	5	5
SPLEEN	(G)	0.4315	0.5160	0.4136	0.3671
		0.0438	0.0832	0.0346	0.0892
		5	5	5	5
	%	0.2418	0.3011 *	0.2314	0.2074
		0.0185	0.0637	0.0151	0.0444
		5	4	5	5
BRAIN	(G)	1.7653	1.7357	1.7191	1.6401
		0.1048	0.0669	0.0706	0.1080
		5	5	5	5
	%	0.9937	0.9539	0.9644	0.9343
		0.1093	0.0749	0.0737	0.0523
		5	4	5	5

KEY: FOR EACH GROUP, VALUES ARE REPORTED AS MEAN, STANDARD DEVIATION AND NUMBER PER GROUP
* - STATISTICALLY DIFFERENT FROM CONTROLS (P</=0.05), ONE WAY ANOVA

GROSS PATHOLOGY REPORT

METHYL CYCLOPROPANECARBOXYLATE

HAEL No.:- 97-0208 **EAN: 007777**

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

AUTHOR

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LABORATORY PROJECT ID

970208I1

STUDY SPONSOR

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REPORT COMPLETION DATE

October 12, 1997

INTRODUCTION

The purpose of this study was to evaluate the effects of the test substance in the rat following four weeks of repeated inhalation exposures. This report covers the results of the necropsy examination conducted during this study.

METHODS:

All rats were fasted overnight, anesthetized with Metofane™, and exsanguinated by severing the posterior vena cava. Necropsies were conducted according to pathology SOP TP 180.

Tissues were fixed by immersion in 10% neutral-buffered formalin for at least 48 hours prior to further processing. The following tissues were collected from each animal: nasal passages, larynx, trachea, lungs, thymus, heart, stomach, duodenum, jejunum, ileum, cecum, colon, liver, kidneys, urinary bladder, adrenal glands, thyroid glands, testes, epididymides, male accessory sex organs, ovaries, Fallopian tubes, uterus, vagina, spleen, mesenteric lymph nodes, cervical lymph nodes, sternum with bone marrow, brain, cervical spinal cord, sciatic nerve, salivary glands, and gross lesions. Trimming procedures were conducted according to pathology SOP TP 210.

Necropsy observations were recorded on an individual necropsy record and at a later time entered into the Automated Animal Toxicology System (AATS) for tabulation of the findings.

GROSS PATHOLOGY:

Male Rats - 1.0 mg/L exposure group: Exposure-related changes in five rats that survived the observation period included minor enlargement (1/5) and minor pallor of the heart (1/5), and minimal or moderate pallor of the liver (4/5).

Incidental findings included a minimal thymus hemorrhage (1/5) and minimal or minor dried porphyrin discharge (2/5) on the nose.

Male Rats - 0.5 mg/L exposure group: Exposure-related changes in five rats that survived the observation period included minimal pallor of the heart (2/5) and a minimal pallor of the liver (1/5).

Incidental findings included a minimal hemorrhage in the cervical lymph nodes (1/5), a minimal focal red discoloration of the lungs (1/5), minimally-reduced size of the spleen (1/5), and a minimal dried porphyrin discharge on the nose (1/5).

Male Rats - 0.1 mg/L exposure group: No exposure-related changes were observed in five rats that survived the observation period.

An incidental finding consisted of moderately enlarged cervical lymph nodes (1/5).

Male Rats - 0 mg/L exposure group: Incidental findings in five rats that survived the observation period consisted of a minor thymus hemorrhage (1/5).

Female Rats - 1.0 mg/L exposure group: Exposure-related changes in five rats that survived the observation period included minor or moderate pallor of the heart (2/5) and minimal to severe pallor of the liver (4/5).

Incidental findings included minimal erosion (1/5) and minimal hemorrhage (1/5) in the glandular gastric mucosa, moderate edema in the gastric serosa (1/5), moderate hydrometra (1/5), minimal or moderate wetness of the inguinal hair by urine (2/5), and minimal or minor dry porphyrin discharge around the nose (4/5).

Female Rats - 0.5 mg/L exposure group: Exposure-related changes in five rats that survived the observation period included minor or moderate pallor of the heart (2/5) and minimal to severe pallor of the liver (5/5).

Incidental findings included minimal hemorrhage in the glandular gastric mucosa (1/5), moderate reduction in the size of the thymus (1/5), minimal dry (1/5) or wet (1/5) urine stain on the inguinal hair, minimal dried porphyrin discharge on the hair of the back (1/5) and around the nose (1/5).

Female Rats - 0.1 mg/L exposure group: Exposure-related changes consisted of minor to severe pallor of the liver (3/5).

Incidental findings included a minimal thymus hemorrhage (1/5), minor hydrometra (1/5), and a minor dry urine stain on the inguinal hair (1/5).

Female Rats - 0.0 mg/L exposure group: Incidental findings in five rats that survived the observation period consisted of a minimal dry urine stain on the inguinal hair (1/5).

COMMENTS:

The control rats of both sexes had no significant lesions. The changes observed in the tissues and organs of the control rats on this study are commonly observed in this age and strain of rat.

Exposure-related lesions were observed in the heart and liver.

Minimal to moderate pallor of the heart was observed in Rats 511, 514, 532, and 535 from the 0.5 mg/L exposure group and in Rats 520, 537, and 540 from the 1.0 mg/L exposure group. In addition, a minor enlargement of the heart was observed in Rat 516 (0.5 mg/L).

Minimal to severe pallor of the liver was observed in Rats 526, 527, and 529 from the 0.1 mg/L exposure group, Rats 512 and 531-535 from the 0.5 mg/L exposure group, and in Rats 516, 518, 519, 520, 537, 538, 539, and 540 from the 1.0 mg/L exposure group.

Incidental findings were observed in the cervical lymph nodes, lungs, thymus, spleen, stomach, uterus, hair, and nose.

The cervical lymph nodes of Rat 506 (0.1 mg/L) were moderately enlarged. Moderate enlargement of the cervical lymph nodes is most likely the result of lymphoid hyperplasia; this change represents a physiological response, occasionally observed in young untreated control rats, rather than a pathologic process.

The cervical lymph nodes of Rat 514 (0.5 mg/L) showed minimal hemorrhage. This lesion was considered an agonal phenomenon occurring shortly before death.

Minimal red discoloration of the lungs was observed in Rat 515 (0.5 mg/L). A red discoloration of this type is frequently the result of congestion and hemorrhage resulting from the euthanasia procedure.

Minimal or minor thymus hemorrhage was observed in Rat 503 (0 mg/L), Rat 530 (0.1 mg/L), and Rat 518 (1.0 mg/L). Thymic hemorrhage was considered an agonal lesion, although it may have also occurred as a result of the dissection of the thymus during necropsy.

A moderate reduction in the size of the thymus was observed in Rat 532 (0.5 mg/L). The cause of this lesion was not determined.

A minimal reduction in the size of the spleen was observed in Rat 511 (0.5 mg/L). The cause of this lesion was not determined.

Minimal hemorrhage was observed in the glandular gastric mucosa of Rat 534 (0.5 mg/L) and Rat 539 (1.0 mg/L). In the absence of necrosis, erosions, and ulcerations, the hemorrhage observed in Rats 534 and 539 were considered to be agonal.

Minimal hemorrhage and minimal erosion of the glandular gastric mucosa and moderate edema of the gastric serosa was observed in Rat 538 (1.0 mg/L). The cause of these lesions was not determined.

Minor or moderate hydrometra was an incidental finding observed in Rat 527 (0.1 mg/L) and Rat 538 (1.0 mg/L). Hydrometra is the dilation of the uterus with an accumulation of ovulatory intraluminal fluid during the estrus cycle of the rat.

Minimal or moderate urinary stain was observed on the inguinal hair of Rat 521 (0.0 mg/L), Rat 529 (0.1 mg/L), Rats 531 and 535 (0.5 mg/L), and Rats 536 and 537 (1.0 mg/L). The cause of this change was not determined.

Minimal or minor dried porphyrin discharges were present on the nose or facial hair of Rat 514 (0.1 mg/L), Rats 534 and 535 (0.5 mg/L), and Rats 517, 520, 536, 537, 538, and 539 (1.0 mg/L). Stress is the most probable cause for porphyrin formation; however, porphyrin discharge is occasionally observed in normal, untreated control rats.

10/12/97
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10/12/97
Reviewed by
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MSV:sji
09/29/97

SUMMARY GROSS PATHOLOGY INCIDENCE TABLE - MALE RATS

GROUP	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
NASAL PASSAGES	5	5	5	5
TRACHEA	5	5	5	5
LARYNX	5	5	5	5
SALIVARY GLANDS	5	5	5	5
STOMACH	5	5	5	5
DUODENUM	5	5	5	5
JEJUNUM	5	5	5	5
ILEUM	5	5	5	5
CECUM	5	5	5	5
COLON	5	5	5	5
URINARY BLADDER	5	5	5	5
THYROID GLANDS	5	5	5	5
CERVICAL LYMPH NODE	5	5	5	5
ENLARGED,NOS	0	1	0	0
HEMORRHAGE	0	0	1	0
MESENTERIC LYMPH NODES	5	5	5	5
BONE MARROW	5	5	5	5
CERVICAL SPINAL CORD	5	5	5	5
SCIATIC NERVE	5	5	5	5
ACCESSORY SEX ORGANS (MALE)	5	5	5	5
HEART	5	5	5	5
PALLOR	0	0	2	1
ENLARGED,NOS	0	0	0	1
LUNGS	5	5	5	5
DISCOLORATION, FOCAL RED	0	0	1	0
LIVER	5	5	5	5
PALLOR	0	0	1	4
KIDNEYS	5	5	5	5
ADRENALS	5	5	5	5
THYMUS	5	5	5	5
HEMORRHAGE	1	0	0	1
SPLEEN	5	5	5	5
SMALL	0	0	1	0
BRAIN	5	5	5	5
TESTES	5	5	5	5
EPIDIDYMIDES	5	5	5	5
NOSE	0	0	1	2
DRIED PORPHYRIN DISCHARGE	0	0	1	2

NUMBERS REPRESENT NUMBER OF TISSUES EXAMINED, OR IN THE CASE OF ABNORMAL FINDINGS, THE NUMBER OF TISSUES WITH EACH ABNORMALITY.

SUMMARY GROSS PATHOLOGY INCIDENCE TABLE - FEMALE RATS

GROUP	0.0 MG/L	0.1 MG/L	0.5 MG/L	1.0 MG/L
NASAL PASSAGES	5	5	5	5
TRACHEA	5	5	5	5
LARYNX	5	5	5	5
SALIVARY GLANDS	5	5	5	5
STOMACH	5	5	5	5
STOMACH, GLANDULAR				
HEMORRHAGE	0	0	1	2
EROSION	0	0	0	1
SEROSA				
EDEMA	0	0	0	1
DUODENUM	5	5	5	5
JEJUNUM	5	5	5	5
ILEUM	5	5	5	5
CECUM	5	5	5	5
COLON	5	5	5	5
URINARY BLADDER	5	5	5	5
THYROID GLANDS	5	5	5	5
CERVICAL LYMPH NODE	5	5	5	5
MESENTERIC LYMPH NODES	5	5	5	5
BONE MARROW	5	5	5	5
CERVICAL SPINAL CORD	5	5	5	5
SCIATIC NERVE	5	5	5	5
OVARIES	5	5	5	5
FALLOPIAN TUBES	5	5	5	5
UTERUS	5	5	5	5
HYDROMETRA	0	1	0	1
VAGINA	5	5	5	5
HEART	5	5	5	5
PALLOR	0	0	2	2
LUNGS	5	5	5	5
LIVER	5	5	5	5
PALLOR	0	3	5	4
KIDNEYS	5	5	5	5
ADRENALS	5	5	5	5
THYMUS	5	5	5	5
HEMORRHAGE	0	1	0	0
SMALL	0	0	1	0
SPLEEN	5	5	5	5
BRAIN	5	5	5	5
HAIR	1	1	2	4
HAIR OF INGUINAL REGION				
HAIRCOAT, DRY URINE STAIN	1	1	1	0
HAIRCOAT, WET BY URINE	0	0	1	2
HAIR OF FACE				
DRIED PORPHYRIN DISCHARGE	0	0	1	4
NOSE	0	0	1	0
DRIED PORPHYRIN DISCHARGE	0	0	1	0

NUMBERS REPRESENT NUMBER OF TISSUES EXAMINED, OR IN THE CASE OF ABNORMAL FINDINGS, THE NUMBER OF TISSUES WITH EACH ABNORMALITY

INDIVIDUAL GROSS PATHOLOGY TABLE - MALE RATS

0.0 MG/L

ANIMAL #	501	502	503	504	505
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE	N	N	N	N	N
MESENTERIC LYMPH NODES	N	N	N	N	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
ACCESSORY SEX ORGANS (MALE)	N	N	N	N	N
HEART	N	N	N	N	N
LUNGS	N	N	N	N	N
LIVER	N	N	N	N	N
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYMUS	N	N		N	N
HEMORRHAGE			2		
SPLEEN	N	N	N	N	N
BRAIN	N	N	N	N	N
TESTES	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N

KEY: N - NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, 1 - MINIMAL, 2 - MINOR, 3 - MODERATE, 4 - SEVERE, P - PRESENT, A - ABSENT, X=NORMAL BUT NOT COLLECTED, * - SEE COMMENT REPORT

INDIVIDUAL GROSS PATHOLOGY TABLE - MALE RATS

	0.1 MG/L				
ANIMAL #	506	507	508	509	510
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE ENLARGED,NOS	3	N	N	N	N
MESENTERIC LYMPH NODES	N	N	N	N	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
ACCESSORY SEX ORGANS (MALE)	N	N	N	N	N
HEART	N	N	N	N	N
LUNGS	N	N	N	N	N
LIVER	N	N	N	N	N
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYMUS	N	N	N	N	N
SPLEEN	N	N	N	N	N
BRAIN	N	N	N	N	N
TESTES	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N

KEY: N - NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, 1 - MINIMAL, 2 - MINOR, 3 - MODERATE, 4 - SEVERE, P - PRESENT, A - ABSENT, X=NORMAL BUT NOT COLLECTED, * - SEE COMMENT REPORT

INDIVIDUAL GROSS PATHOLOGY TABLE - MALE RATS

	0.5 MG/L				
ANIMAL #	511	512	513	514	515
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE	N	N	N		N
HEMORRHAGE				1	
MESENTERIC LYMPH NODES	N	N	N	N	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
ACCESSORY SEX ORGANS (MALE)	N	N	N	N	N
HEART		N	N		N
PALLOR	1			1	
LUNGS	N	N	N	N	
DISCOLORATION, FOCAL RED					1
LIVER	N		N	N	N
PALLOR		1			
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYMUS	N	N	N	N	N
SPLEEN		N	N	N	N
SMALL	1				
BRAIN	N	N	N	N	N
TESTES	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N
NOSE					
DRIED PORPHYRIN DISCHARGE				1	

KEY: N - NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, 1 - MINIMAL, 2 - MINOR, 3 - MODERATE, 4 - SEVERE, P - PRESENT, A - ABSENT, X=NORMAL BUT NOT COLLECTED, * - SEE COMMENT REPORT

INDIVIDUAL GROSS PATHOLOGY TABLE - MALE RATS

	1.0 MG/L				
ANIMAL #	516	517	518	519	520
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE	N	N	N	N	N
MESENTERIC LYMPH NODES	N	N	N	N	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
ACCESSORY SEX ORGANS (MALE)	N	N	N	N	N
HEART		N	N	N	
ENLARGED,NOS	2				
PALLOR					2
LUNGS	N	N	N	N	N
LIVER		N			
PALLOR	1		3	1	1
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYMUS	N	N		N	N
HEMORRHAGE			1		
SPLEEN	N	N	N	N	N
BRAIN	N	N	N	N	N
TESTES	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N
NOSE					
DRIED PORPHYRIN DISCHARGE		2			1

KEY: N - NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, 1 - MINIMAL, 2 - MINOR, 3 - MODERATE, 4 - SEVERE, P - PRESENT, A - ABSENT, X=NORMAL BUT NOT COLLECTED, * - SEE COMMENT REPORT

INDIVIDUAL GROSS PATHOLOGY TABLE - FEMALE RATS

0.0 MG/L

ANIMAL #	521	522	523	524	525
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE	N	N	N	N	N
MESENTERIC LYMPH NODES	N	N	N	N	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
OVARIES	N	N	N	N	N
FALLOPIAN TUBES	N	N	N	N	N
UTERUS	N	N	N	N	N
VAGINA	N	N	N	N	N
HEART	N	N	N	N	N
LUNGS	N	N	N	N	N
LIVER	N	N	N	N	N
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYMUS	N	N	N	N	N
SPLEEN	N	N	N	N	N
BRAIN	N	N	N	N	N
HAIR					
HAIR OF INGUINAL REGION					
HAIRCOAT, DRY URINE STAIN	1				

KEY: N - NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, 1 - MINIMAL, 2 - MINOR, 3 - MODERATE, 4 - SEVERE, P - PRESENT, A - ABSENT, X=NORMAL BUT NOT COLLECTED, * - SEE COMMENT REPORT

INDIVIDUAL GROSS PATHOLOGY TABLE - FEMALE RATS

	0.1 MG/L				
ANIMAL #	526	527	528	529	530
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N	N	N	N
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE	N	N	N	N	N
MESENTERIC LYMPH NODES	N	N	N	N	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
OVARIES	N	N	N	N	N
FALLOPIAN TUBES	N	N	N	N	N
UTERUS	N		N	N	N
HYDROMETRA		2			
VAGINA	N	N	N	N	N
HEART	N	N	N	N	N
LUNGS	N	N	N	N	N
LIVER			N		N
PALLOR	4	2		3	
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
*THYMUS	N	N	N	N	
HEMORRHAGE					1
SPLEEN	N	N	N	N	N
BRAIN	N	N	N	N	N
HAIR					
HAIR OF INGUINAL REGION					
HAIRCOAT, DRY URINE STAIN				2	

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INDIVIDUAL GROSS PATHOLOGY TABLE - FEMALE RATS

	0.5 MG/L				
ANIMAL #	531	532	533	534	535
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N	N		N
STOMACH, GLANDULAR					
HEMORRHAGE				1	
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE	N	N	N	N	N
MESENTERIC LYMPH NODES	N	N	N	N*	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
OVARIES	N	N	N	N	N
FALLOPIAN TUBES	N	N	N	N	N
UTERUS	N	N	N	N	N
VAGINA	N	N	N	N	N
HEART	N		N	N	
PALLOR		3			2
LUNGS	N	N	N	N	N
LIVER					
PALLOR	3	4	2	1	4
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYMUS	N		N	N	N
SMALL		3			
SPLEEN	N	N	N	N	N
BRAIN	N	N	N	N	N
HAIR					
HAIR OF INGUINAL REGION					
HAIRCOAT, WET BY URINE	1				
HAIRCOAT, DRY URINE STAIN					1
HAIR OF FACE					
DRIED PORPHYRIN DISCHARGE					1
NOSE					
DRIED PORPHYRIN DISCHARGE				1	

KEY: N - NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, 1 - MINIMAL, 2 - MINOR, 3 - MODERATE, 4 - SEVERE, P - PRESENT, A - ABSENT, X=NORMAL BUT NOT COLLECTED, * - SEE COMMENT REPORT

INDIVIDUAL GROSS PATHOLOGY TABLE - FEMALE RATS

1.000 MG/L

ANIMAL #	536	537	538	539	540
DAYS ON TEST	30	30	30	30	30
NASAL PASSAGES	N	N	N	N	N
TRACHEA	N	N	N	N	N
LARYNX	N	N	N	N	N
SALIVARY GLANDS	N	N	N	N	N
STOMACH	N	N			N
STOMACH, GLANDULAR					
EROSION			1		
HEMORRHAGE			1	1	
SEROSA					
EDEMA			3		
DUODENUM	N	N	N	N	N
JEJUNUM	N	N	N	N	N
ILEUM	N	N	N	N	N
CECUM	N	N	N	N	N
COLON	N	N	N	N	N
URINARY BLADDER	N	N	N	N	N
THYROID GLANDS	N	N	N	N	N
CERVICAL LYMPH NODE	N	N	N	N	N
MESENTERIC LYMPH NODES	N	N	N	N	N
BONE MARROW	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N
SCIATIC NERVE	N	N	N	N	N
OVARIES	N	N	N	N	N
FALLOPIAN TUBES	N	N	N	N	N
UTERUS	N	N		N	N
HYDROMETRA			3		
VAGINA	N	N	N	N	N
HEART	N		N	N	
PALLOR		3			2
LUNGS	N	N	N	N	N
LIVER	N				
PALLOR		4	4	1	3
KIDNEYS	N	N	N	N	N
ADRENALS	N	N	N	N	N
THYMUS	N	N	N	N	N
SPLEEN	N	N	N	N	N
BRAIN	N	N	N	N	N
HAIR					
HAIR OF INGUINAL REGION					
HAIRCOAT, WET BY URINE	1	3			
HAIR OF FACE					
DRIED PORPHYRIN DISCHARGE	1	1	2	1	

KEY: N - NORMAL AND TISSUE COLLECTED FOR HISTOPATHOLOGY, 1 - MINIMAL, 2 - MINOR, 3 - MODERATE, 4 - SEVERE, P - PRESENT, A - ABSENT, X=NORMAL BUT NOT COLLECTED, * - SEE COMMENT REPORT

GROSS PATHOLOGY COMMENT REPORT

DAY	DOSE LEVEL	ANIMAL #	COMMENT
31	0.1 MG/L	529	THE THYMUS WAS LOST AFTER WEIGHING.

CONSULTANTS IN VETERINARY PATHOLOGY

P.O. Box 68
Murrysville, PA 15668-0068
Phone: (724) 733-5154
Fax: (724) 733-3032

METHYL CYCLOPROPANECARBOXYLATE
SYNONYM: MCPC

HAEL NO.: 97-0208 **EAN: 007777**
CAS No.: 002868-37-3 **PM No. 15858-00**

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

PERFORMING LABORATORY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

LABORATORY PROJECT ID

970208I1

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-0431

Sponsor's Representative:
Karen R. Miller, Ph.D.

(55 Pages)

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Those portions of this study which were performed at the laboratory of Consultants in Veterinary Pathology were conducted in compliance with the appropriate sections of the following Good Laboratory Practice Standards: United States Environmental Protection Agency, Toxic Substances Control Act, 40 CFR Part 792 and Annex 2, Organisation for Economic Cooperation and Development, Guidelines for Testing of Chemicals [C(81)30 (Final)].

Robert H. Garman 5-15-98

Robert H. Garman, DVM — Date
Diplomate, ACVP
Consultant Pathologist

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

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A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

SUMMARY

Microscopic evaluations were performed on a variety of tissues from 20 male and 20 female Sprague-Dawley® [SAS:VAF®(SD)] rats which had been on a four-week study designed to evaluate the toxicity of the test chemical when administered *via* daily repeated inhalation exposures (six hours/day and five days/week, excluding holidays, for four weeks and on Monday and Tuesday of the fifth week). Five rats/sex were exposed to the test chemical at concentrations of either 0, 0.1, 0.5, or 1.0 mg/L. Microscopic examinations were performed on a protocol-specified selection of tissues from the rats in the high concentration and control groups. Sections of liver, heart, testes, and epididymides were examined microscopically from the male rats in the 0.1 and 0.5 mg/L group. For the female rats in the 0.1 and 0.5 mg/L groups, microscopic evaluations were performed on sections of the liver, heart, and sternal bone marrow. Sections of tissues having gross abnormalities at the time of necropsy were examined from all of the rats on study.

Exposure-related microscopic findings included the following: myocarditis, muscle fiber vacuolation, and muscle fiber (myocyte) degeneration within the heart (present in both sexes in all three test chemical exposure groups); hepatocellular vacuolation (present with increased severity *vs.* the control group in all three exposure groups); degeneration of spermatids and/or spermatozoa within the testes and epididymides of the rats in the 1.0 mg/L group. There was a mild decrease in cellularity within the sternal bone marrow of three female rats in the 1.0 mg/L group and one female in the 0.5 mg/L group, as well as a minimal decrease in cellularity in the sternal marrow of one female in the 0.1 mg/L group. Based on the liver and cardiac alterations, a no effect level is not present on this study.

INTRODUCTION

This report details the materials and methods followed for the histopathology portion of this study and includes the results of the microscopic evaluations performed on tissues from 20 male and 20 female Sprague-Dawley® [SAS:VAF®(SD)] rats which had been on a four-week inhalation toxicity study. Inhalation exposures were for six hours/day and five days/week, excluding holidays, for four weeks and on Monday and Tuesday of the fifth week. Five rats/sex were exposed to the test chemical at concentrations of either 0, 0.1, 0.5, or 1.0 mg/L.

MATERIALS AND METHODS

Study Overview and Group Assignments:

A protocol-specified selection of tissues were processed and microscopically evaluated from the 20 rats/sex in the control and 1.0 mg/L exposure groups. Target tissues (liver, heart, testes and epididymides for the males; liver, heart and bone marrow for the females) were microscopically evaluated from the rats in the 0.1 and 0.5 mg/L exposure groups. Tissues having gross lesions at the time of necropsy were examined for all rats on study. The necropsy examinations on these rats had been conducted at the Performing Laboratory which also conducted the in-life phase of this study.

Histotechnology Procedures:

The tissues were received in formalin from the Performing Laboratory. After checking each bottle label against the animal numbers on the individual animal Necropsy Report forms, the tissues were trimmed according to standard procedures and processed for embedding in paraffin. As the tissues were trimmed, the abbreviated gross findings listed on the individual animal Necropsy Report forms were also checked and, if additional gross observations were made, these were recorded on the Consultants In Veterinary Pathology Histology Processing Sheet. All grossly visible lesions were trimmed for microscopic evaluation.

The block list for the tissues evaluated from the high dose and control group rats is as follows:

Male and Female Rats:

Block 1: Lungs

Block 2: Heart, Liver

Block 3: Spleen, Adrenals

Block 4: Trachea, Larynx, Thyroids

Block 5: Kidneys, Urinary Bladder

Block 6: Stomach, Duodenum, Jejunum, Ileum, Cecum, Colon

Block 7: Salivary Glands, Thymus, Mesenteric Lymph Nodes

Block 9: Brain

Block 10: Cervical Spinal Cord, Cervical (Submandibular) Lymph Node

Block 11: Sciatic Nerve

Block 12: Sternum (with Bone Marrow)

Block 13: Nasal Passages

Additional Blocks for Male Rats:

Block 8: Testicle, Epididymis, Prostate

Block 8A: Testicle, Epididymis, Seminal Vesicles, Coagulating Glands

Additional Blocks for Female Rats:

Block 8: Ovaries, Vagina, Uterus, and Fallopian tubes

When target tissues or tissues with gross alterations were processed from the rats in 0.1 and 0.5 mg/L exposure groups, the same respective tissue block numbers (from the above list) were used. However, because tissues with gross lesions were processed first (*i.e.* before the target organs were determined), some rats have multiple blocks with the same number but which bear additional letter designations. (For example, the liver may be in Block #2 and the heart in Block #2A or vica versa, rather than both organs being in Block #2). If a tissue not included in the standard tissue list had been found to have a gross lesion, Block 14 would have been used.

The tissue blocks were sectioned with a rotary microtome set at a section thickness of four micrometers, excepting for those blocks containing the brain slices, which were sectioned at five micrometers. The resulting sections were stained with hematoxylin and eosin. If the initial microscopic examinations revealed a tissue to be missing, a recut was prepared from the tissue block.

Microscopic Evaluations:

Microscopic findings were recorded and tabulated using a PC-based computer program (GLPATH; Great Laboratory ProgramS®). The gross observations made at the Sponsor's laboratory were also entered into this program so that gross/microscopic correlates could be made whenever possible. If any additional gross observations had been recorded on the Histology Processing Sheets during the trimming of the tissues, these additional findings would also have been entered into the computer data base. All microscopic lesions were assigned one of five severity grades (*viz.* minimal, mild, moderate, marked, and severe). The distribution pattern of each lesion (focal, multifocal, or diffuse) was also assigned. These distributions will be found with the individual animal data (Tables 3 and 4) rather than in the microscopic lesion summary tables (Tables 1 and 2). Correlates were made, whenever possible, between the gross observations and the appropriate microscopic changes.

RESULTS AND DISCUSSION

Graded microscopic summary findings for the male rats necropsied after four weeks on study are presented in Table 1, and summary microscopic findings for the female rats are presented in Table 2. The individual animal microscopic findings for these rats are presented in Table 3 for the male rats and in Table 4 for the female rats. Tables of gross findings are not included in this report. No rats died on study or were sacrificed early because of moribundity.

Exposure-related increases in the frequencies of microscopic lesions or tissue alterations were found for the following tissues:

Heart:

A triad of exposure-related tissue alterations were present in the heart, these including vacuolation of the cytoplasm of the myocardial muscle cells (**myocyte vacuolation**), **myocarditis**, and **muscle fiber degeneration**. For the male rats, both the frequencies and severities of myocyte vacuolation were found to be increased in relation to the test chemical concentration, but the only rat graded as "moderate" for this change was in the 0.5 mg/L group. For the female rats, all rats in each test chemical-exposed group had myocyte vacuolation, and there was no apparent relationship between the severity of this change and the test chemical concentration. For animals with grades of minimal or mild for myocyte vacuolation, the intracytoplasmic vacuoles were small in size (usually about 2 - 3 micrometers in diameter) and finely distributed throughout the sarcoplasm. For those animals with grades of moderate, at least a small percentage of the vacuoles were slightly larger in size (*viz.* 5 - 7 micrometers or slightly larger). The distribution of myocyte vacuolation was often patchy but, for all animals, was most prominent within the ventricular muscles (left ventricle, right ventricle, and interventricular septum).

Myocarditis was present in all of the male rats exposed to the test chemical, as well as in all exposed female rats excepting for three high concentration group animals. As in the case of myocyte vacuolation in the female rats, there was no increase in severity of myocarditis with test chemical concentration. The lesion classified as myocarditis in this study is characterized by intermyocytic and perivascular infiltrates of "round cells" with active-appearing nuclei. Although the nuclear features of these cells most closely resemble those of activated lymphocytes, some of these nuclei could belong to activated connective tissue cells within the endomysium. The appearance of this lesion is somewhat suggestive of a hypersensitivity myocarditis, but lacks a component of

eosinophils. Only small numbers of neutrophils are present within the foci of myocarditis. When present, these neutrophils are generally associated with necrotic myocytes.

Within some of the foci of myocarditis, individual degenerative or necrotic cardiac myocytes are present (classified in this study as "muscle fiber degeneration"). These muscle cells have brightly eosinophilic hyalinized cytoplasm and darkly-stained pyknotic or fragmented nuclei. Muscle fiber degeneration was of only minimal degree for the majority of the test chemical-exposed rats but was classified as being mild in degree for a few rats. (NOTE: Even in those hearts classified as "mild" for muscle fiber degeneration, the pathologist must search diligently for these necrotic fibers and must also be careful to distinguish these cells from myocytes characterized by an artefactual increase in sarcoplasmic eosinophilia.) As for the other microscopic cardiac alterations, there was no clear effect of concentration on the severity of cardiac muscle fiber degeneration. In fact, for the female rats, only one rat in the 1.0 mg/L group had evidence of such muscle fiber degeneration.

Additional exposure-related cardiac findings were restricted to the male rats in the 0.5 mg/L group. These findings included three male rats with atrial thrombosis, one with endocarditis and two with epicarditis. One of the male rats had both epicarditis and endocarditis, and one had both epicarditis and atrial thrombosis. Therefore, a total of four out of the five male rats in the 0.5 mg/L group had at least one of these three lesions. The male rat in the 0.5 mg/L group which was classified as having a moderate degree of myocarditis also had a marked degree of atrial thrombosis, and this myocarditis was primarily neutrophilic in type and, based on its distribution, was secondary to the presence of the thrombus (although a small focus of mononuclear cell myocarditis was also found within the wall of one of the ventricles). The extent to which the atrial thrombi in the 0.5 mg/L group male rats may have been related to the other cardiac alterations of vacuolation, myocarditis, or muscle fiber degeneration is not clear, because these latter three processes primarily affected the ventricular musculature.

Liver:

The only exposure-related microscopic finding in the liver is that of **hepatocellular cytoplasmic vacuolation**. Hepatocellular vacuolation usually indicates the presence of increased amounts of fat within the hepatocytes. (This could be confirmed by examination of cryostatically prepared sections treated with fat stains.) Hepatocellular vacuolation may occur as a result of hepatotoxicity or may be secondary to increase mobilization of body fat (as in anorexia). The pattern of vacuolation present in the livers of the rats on this study is of the microvesicular type and characterized by small-sized intracytoplasmic vacuoles which often swell the hepatocyte somas but leave the nuclei in a central location. The cytoplasmic vacuoles range from approximately 2 - 7 micrometers in diameter and are clear (empty) in appearance.

A greater degree of "background" hepatocellular vacuolation was present within the liver sections of the control female rats, so a statistically significant increase in the frequency of vacuolation is not present for this sex in Table 2. However, all of the exposed female rats had greater severities of hepatocellular vacuolation than did the control females. Furthermore, hepatocellular vacuolation in the male rats was restricted to those rats which were exposed to the test chemical. As in the case of the cardiac lesions, there is no clear evidence of increased severity of hepatocellular vacuolation with increased concentration of the test chemical.

Testes and Epididymides:

Although the testes of the test chemical-exposed rats were relatively normal in appearance, slightly increased numbers of degenerative cells (classified as "**spermatid degeneration**") resided within the lumens of small numbers of seminiferous tubules within the testes of the 1.0 mg/L group rats. The presence of an increased degree of spermatid or spermatozoal degeneration is confirmed by the presence of large numbers of such degenerative cells within many of the lumens of the epididymal tubules of the 1.0 mg/L group rats. These changes were limited to the rats in the 1.0 mg/L group. While the mechanism of this hightened degree of

individual apoptotic cell degeneration is not known, there was no evidence, microscopically, of degeneration of spermatogonia and also no evidence of seminiferous tubule atrophy. Although the epididymal tubules of the 1.0 mg/L group rats contain moderate numbers of degenerative cells, these tubules also contain large numbers of normal-appearing spermatozoa, and the tubules are, otherwise, normal in appearance.

Bone Marrow:

Minimal to mild degrees of sternal bone marrow hypoplasia were present in one female in each of the 0.1 and 0.5 mg/L groups, as well as in three of the five females in the 1.0 mg/L group. An application of the term "hypoplasia" reflects the presence of slightly lesser numbers of erythropoietic and myelopoietic cells within these marrows than are present in the control females, but the degree of marrow cellularity may possibly still be within the spectrum of normal. Accurate quantization of the cell types present within the marrow sections is difficult because of the presence of cytologic artifact related to the decalcification procedure. However, evaluation of marrow smears could possibly provide additional information which might either corroborate or invalidate a hypothesized test chemical effect on the marrow.

Kidney:

The only other microscopic alteration which may possibly be exposure-related is that of vacuolation of the cytoplasm of the proximal convoluted tubules of the kidney (documented as "**vacuolation of renal tubules**"). This alteration was not noted on the original examination of the kidneys. However, after the initial exam, it was learned that there was an exposure-related effect on kidney weights. The renal sections were, therefore, reevaluated, and slightly greater than expected degrees of cytoplasmic vacuolation were noted in three out of five of the 1.0 mg/L group female rats. No such differences were noted for the male rat kidneys, however. It is important to note that minimal degrees of cytoplasmic vacuolation are normally present within the cytoplasm of the proximal convoluted tubules of control rat kidneys (and were also present in this study). Furthermore, it is quite possible that the mild to moderate degree of vacuolation present in the 1.0 mg/L group female kidneys may either be within the spectrum of normal or may represent a cytologic alteration reflective of postmortem autolytic change. Kidney sections have not been examined from the 0.1 and 0.5 mg/L group rats.

CONCLUSION

Multiple exposure-related microscopic findings include myocarditis, muscle fiber (myocyte) vacuolation, and muscle fiber degeneration within the heart (present in both sexes in all three test chemical exposure groups), hepatocellular vacuolation (present in both sexes with increased severity in all three exposure groups), degeneration of spermatids and/or spermatozoa within the testes and epididymides of the male rats in the 1.0 mg/L group, and decreased cellularity of the sternal bone marrow in three female rats in the 1.0 mg/L group, one female in the 0.5 mg/L group, and one female in the 0.1 mg/L group. Based on the liver and cardiac alterations, a no effect level is not present on this study.

Robert H. Garman 5-15-98

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Date

A|CVP 97-2; 5/14/98

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
HEART					
Number of Tissues Examined		5	5	5	5
Microscopically Normal		4	0	0	0
No. With Microscopic Diagnoses		1	5	5	5
MONONUCLEAR CELL INFILTRATE(S)		1	0	0	0
	mild	1	-	-	-
MYOCYTE VACUOLATION		0	1	4a	5b
	minimal	-	1	3	-
	mild	-	-	-	5
	moderate	-	-	1	-
ATRIAL THROMBOSIS		0	0	3	0
	mild	-	-	1	-
	moderate	-	-	1	-
	marked	-	-	1	-
ENDOCARDITIS		0	0	1	0
	mild	-	-	1	-
MYOCARDITIS		0	5b	5b	5b
	minimal	-	4	-	1
	mild	-	1	4	4
	moderate	-	-	1	-
EPICARDITIS		0	0	2	0
	minimal	-	-	1	-
	mild	-	-	1	-
MUSCLE FIBER DEGENERATION		0	3	5b	5b
	minimal	-	1	5	4
	mild	-	2	-	1

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
a - Significantly different from GROUP 1 at P<=0.05
b - Significantly different from GROUP 1 at P<=0.01

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

		GROUP:	1	2	3	4
Number of animals included			5	5	5	5
SALIVARY GL						
Number of Tissues Examined			5	0	0	5
No. With Microscopic Diagnoses			5	-	-	5
VACUOLATION OF ACINAR AND DUCT CELLS			5	-	-	5
	minimal		5	-	-	4
	mild		-	-	-	1
STOMACH						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			2	-	-	1
No. With Microscopic Diagnoses			3	-	-	4
GLAND ECTASIA			2	-	-	2
	minimal		1	-	-	-
	mild		1	-	-	2
EDEMA			1	-	-	3
	minimal		1	-	-	3
GASTRITIS			3	-	-	2
	minimal		3	-	-	2
LIVER						
Number of Tissues Examined			5	5	5	5
Microscopically Normal			4	1	0	0
No. With Microscopic Diagnoses			1	4	5	5
HEPATOCELLULAR CYTOPLASMIC VACUOLATION			0	4a	4a	5b
	minimal		-	-	1	2
	mild		-	4	2	2
	moderate		-	-	1	1
APOPTOTIC HEPATOCYTES			0	0	0	1
	minimal		-	-	-	1

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
a = Significantly different from GROUP 1 at P<=0.05
b = Significantly different from GROUP 1 at P<=0.01

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
LIVER(continued)					
INFLAMMATORY CELL INFILTRATE(S)		0	0	1	0
	mild	-	-	1	-
TRIADITIS		1	3	1	1
	minimal	1	2	-	1
	mild	-	1	1	-
BILE DUCT HYPERPLASIA		0	1	1	0
	minimal	-	1	1	-
FIBROSIS, CAPSULE		0	1	0	0
	mild	-	1	-	-
DUODENUM					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
JEJUNUM					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
ILEUM					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		3	-	-	4
No. With Microscopic Diagnoses		2	-	-	1
LYMPHOID HYPERPLASIA		2	-	-	1
	mild	2	-	-	1
CECUM					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		4	-	-	5
No. With Microscopic Diagnoses		1	-	-	0
EDEMA		1	-	-	0
	minimal	1	-	-	-

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

		GROUP:	1	2	3	4
Number of animals included			5	5	5	5
CECUM(continued)						
LYMPHOID HYPERPLASIA			1	-	-	0
	mild		1	-	-	-
COLON						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			5	-	-	5
THYROID GL						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			5	-	-	5
ADRENAL GL						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			5	-	-	5
SPLEEN						
Number of Tissues Examined			5	0	1	5
Microscopically Normal			5	-	1	5
LYMPH ND, CERVICAL						
Number of Tissues Examined			5	1	1	5
Microscopically Normal			2	0	0	2
No. With Microscopic Diagnoses			3	1	1	3
HEMORRHAGE			0	0	0	1
	mild		-	-	-	1
LYMPHOID HYPERPLASIA			3	1	1	3
	mild		1	-	-	-
	moderate		-	-	1	3
	marked		1	1	-	-
	severe		1	-	-	-
LYMPH ND, MES						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			5	-	-	5

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
THYMIC REGION					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		3	-	-	3
No. With Microscopic Diagnoses		2	-	-	2
LYMPHOCYTE DEGENERATION		2	-	-	2
	minimal	2	-	-	1
	mild	-	-	-	1
BONE MARROW					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
BONE, STERNUM					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
BRAIN					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
SPINAL CORD					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
NERVE, SCIATIC					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
TESTES					
Number of Tissues Examined		5	5	5	5
Microscopically Normal		5	5	5	0
No. With Microscopic Diagnoses		0	0	0	5
SPERMATID DEGENERATION		0	0	0	5b
	minimal	-	-	-	5

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
b - Significantly different from GROUP 1 at P<=0.01

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
EPIDIDYMIDES					
Number of Tissues Examined		5	5	5	5
Microscopically Normal		4	5	5	0
No. With Microscopic Diagnoses		1	0	0	5
DEGENERATING CELLS WITHIN TUBULE LUMENS		1	0	0	5a
	minimal	1	-	-	-
	mild	-	-	-	4
	moderate	-	-	-	1
EPIDIDYMITIS		1	0	0	0
	mild	1	-	-	-
SEMINAL VESICLE					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
COAGULATING GL					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
PROSTATE					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		2	-	-	4
No. With Microscopic Diagnoses		3	-	-	1
GLAND ECTASIA		1	-	-	0
	mild	1	-	-	-
PROSTATITIS		3	-	-	1
	mild	2	-	-	1
	moderate	1	-	-	-
NOSE/TURBINATES					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
a = Significantly different from GROUP 1 at P<=0.05

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
LARYNX					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		2	-	-	3
No. With Microscopic Diagnoses		3	-	-	2
LARYNGITIS		3	-	-	2
	minimal	2	-	-	1
	mild	1	-	-	1
TRACHEA					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
LUNGS					
Number of Tissues Examined		5	0	1	5
Microscopically Normal		0	-	0	1
No. With Microscopic Diagnoses		5	-	1	4
ALVEOLAR HISTIOCYTOSIS		1	-	0	1
	minimal	1	-	-	1
HEMORRHAGE		0	-	1	1
	minimal	-	-	-	1
	mild	-	-	1	-
MINERALIZATION, PULMONARY VESSEL(S)		2	-	0	2
	minimal	2	-	-	2
OSSIFICATION, METAPLASTIC		1	-	0	0
	minimal	1	-	-	-
ATELECTASIS		5	-	1	3
	minimal	5	-	1	2
	mild	-	-	-	1
PNEUMONITIS, INTERSTITIAL		0	-	1	0
	mild	-	-	1	-

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 1
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR MALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
KIDNEYS					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
URINARY BLADDER					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		4	-	-	5
No. With Microscopic Diagnoses		1	-	-	0
EDEMA		1	-	-	0
	mild	1	-	-	-

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 2
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR FEMALE RATS

		GROUP:	1	2	3	4
Number of animals included			5	5	5	5
HEART						
Number of Tissues Examined			5	5	5	5
Microscopically Normal			5	0	0	0
No. With Microscopic Diagnoses			0	5	5	5
MYOCYTE VACUOLATION			0	5b	5b	5b
	minimal		-	1	-	-
	mild		-	2	2	4
	moderate		-	2	3	1
MYOCARDITIS			0	5b	5b	2
	minimal		-	-	3	1
	mild		-	4	2	1
	moderate		-	1	-	-
MUSCLE FIBER DEGENERATION			0	5b	4a	1
	minimal		-	4	4	1
	mild		-	1	-	-
SALIVARY GL						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			5	-	-	5
STOMACH						
Number of Tissues Examined			5	0	1	5
Microscopically Normal			3	-	0	1
No. With Microscopic Diagnoses			2	-	1	4
EDEMA			2	-	1	4
	minimal		1	-	-	1
	mild		1	-	-	2
	moderate		-	-	1	1
GASTRITIS			2	-	1	4
	minimal		2	-	1	3
	mild		-	-	-	1

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
a - Significantly different from GROUP 1 at P<=0.05
b - Significantly different from GROUP 1 at P<=0.01

TABLE 2
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR FEMALE RATS

		GROUP:	1	2	3	4
Number of animals included			5	5	5	5
STOMACH(continued)						
MUCOSAL EROSION(S)			0	-	1	1
	mild		-	-	-	1
	moderate		-	-	1	-
LIVER						
Number of Tissues Examined			5	5	5	5
No. With Microscopic Diagnoses			5	5	5	5
HEPATOCELLULAR CYTOPLASMIC VACUOLATION			5	5	5	5
	minimal		4	-	-	-
	mild		1	-	-	-
	moderate		-	3	1	2
	marked		-	2	4	3
APOPTOTIC HEPATOCYTES			1	0	0	0
	minimal		1	-	-	-
INFLAMMATORY CELL INFILTRATE(S)			1	1	2	1
	minimal		1	1	2	1
TRIADITIS			0	0	1	0
	minimal		-	-	1	-
BILE DUCT HYPERPLASIA			1	1	2	0
	minimal		1	1	2	-
DUODENUM						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			5	-	-	5
JEJUNUM						
Number of Tissues Examined			5	0	0	5
Microscopically Normal			5	-	-	5

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 2
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR FEMALE RATS

GROUP:	1	2	3	4
Number of animals included	5	5	5	5
ILEUM				
Number of Tissues Examined	5	0	0	5
Microscopically Normal	5	-	-	5
CECUM				
Number of Tissues Examined	5	0	0	5
Microscopically Normal	5	-	-	5
COLON				
Number of Tissues Examined	5	0	0	5
Microscopically Normal	5	-	-	5
THYROID GL				
Number of Tissues Examined	5	0	0	5
Microscopically Normal	5	-	-	5
ADRENAL GL				
Number of Tissues Examined	5	0	0	5
Microscopically Normal	5	-	-	5
SPLEEN				
Number of Tissues Examined	5	0	0	5
Microscopically Normal	5	-	-	5
LYMPH ND, CERVICAL				
Number of Tissues Examined	5	0	0	4
Microscopically Normal	1	-	-	1
No. Not Examined Microscopically	-	-	-	1
No. With Microscopic Diagnoses	4	-	-	3
LYMPHADENITIS	0	-	-	1
mild	-	-	-	1
LYMPHOID HYPERPLASIA	4	-	-	2
mild	4	-	-	2

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 2
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR FEMALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
LYMPH ND, MES					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
THYMIC REGION					
Number of Tissues Examined		5	1	1	5
Microscopically Normal		3	0	0	4
No. With Microscopic Diagnoses		2	1	1	1
HEMORRHAGE		0	1	1	0
	mild	-	1	1	-
LYMPHOCYTE DEGENERATION		2	0	0	1
	minimal	2	-	-	1
BONE MARROW					
Number of Tissues Examined		5	5	5	5
Microscopically Normal		5	4	4	2
No. With Microscopic Diagnoses		0	1	1	3
HYPOPLASIA		0	1	1	3
	minimal	-	1	-	-
	mild	-	-	1	3
BONE, STERNUM					
Number of Tissues Examined		5	5	5	5
Microscopically Normal		0	3	2	0
No. With Microscopic Diagnoses		5	2	3	5
CARTILAGE DEGENERATION		5	2	3	5
	minimal	1	2	3	3
	mild	4	-	-	2
BRAIN					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 2
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR FEMALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
SPINAL CORD					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
NERVE, SCIATIC					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
OVARIES					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
OVIDUCT					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
UTERUS					
Number of Tissues Examined		5	1	0	5
Microscopically Normal		3	0	-	4
No. With Microscopic Diagnoses		2	1	-	1
HEMOSIDEROSIS		1	0	-	0
	minimal	1	-	-	-
VASCULAR ECTASIA		1	0	-	0
	mild	1	-	-	-
LUMEN ECTASIA		1	1	-	1
	mild	1	-	-	-
	marked	-	1	-	1
VAGINA					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	4
No. With Microscopic Diagnoses		0	-	-	1
VAGINITIS		0	-	-	1
	mild	-	-	-	1

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 2
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR FEMALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
NOSE/TURBINATES					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
LARYNX					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
TRACHEA					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		5	-	-	5
LUNGS					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		1	-	-	1
No. With Microscopic Diagnoses		4	-	-	4
ALVEOLAR HISTIOCYTOSIS		1	-	-	2
	minimal	1	-	-	2
HEMORRHAGE		1	-	-	1
	minimal	1	-	-	1
MINERALIZATION, PULMONARY VESSEL(S)		1	-	-	1
	minimal	1	-	-	1
ATELECTASIS		2	-	-	2
	minimal	1	-	-	-
	mild	1	-	-	2
KIDNEYS					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		2	-	-	1
No. With Microscopic Diagnoses		3	-	-	4
MINERALIZATION		3	-	-	2
	minimal	1	-	-	1
	mild	2	-	-	1

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 2
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
SUMMARY OF MICROSCOPIC DIAGNOSES FOR FEMALE RATS

GROUP:		1	2	3	4
Number of animals included		5	5	5	5
KIDNEYS(continued)					
VACUOLATION OF RENAL TUBULES		0	-	-	3
	mild	-	-	-	2
	moderate	-	-	-	1
LYMPHOID CELL INFILTRATE(S)		1	-	-	0
	mild	1	-	-	-
NEPHRITIS, INTERSTITIAL		1	-	-	0
	minimal	1	-	-	-
URINARY BLADDER					
Number of Tissues Examined		5	0	0	5
Microscopically Normal		3	-	-	3
No. With Microscopic Diagnoses		2	-	-	2
EDEMA		1	-	-	2
	mild	1	-	-	-
	moderate	-	-	-	2
LYMPHOCYTIC INFILTRATE(S)		1	-	-	0
	moderate	1	-	-	-

Group Legend: 1 is 0 mg/L, 2 is 0.1 mg/L, 3 is 0.5 mg/L, 4 is 1.0 mg/L
Statistics performed using Fisher's exact (1-tail)
None significantly different from GROUP 1

TABLE 3
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
INDIVIDUAL ANIMAL DIAGNOSES FOR MALE RATS

Group: 0 mg/L

ANIMAL ID: 501 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
HEART (microscopic)		
	MONONUCLEAR CELL INFILTRATE(S), focal, mild	
	Comment: Comprised of moderate numbers of lymphoblasts, suggesting that the process in the cervical l.n. may represent lymphoma.	
SALIVARY GL (microscopic)		
	VACUOLATION OF ACINAR AND DUCT CELLS, multifocal, minimal	
LYMPH ND, CERVICAL (microscopic)		
	LYMPHOID HYPERPLASIA, diffuse, severe	
	Comment: Possibly an early stage lymphoma, although a very marked degree of plasmacytopoiesis is present.	
LARYNX (microscopic)		
	LARYNGITIS, multifocal, mild	
LUNGS (microscopic)		
	ATELECTASIS, multifocal, minimal	

The following tissues/anatomic sites are microscopically normal:

STOMACH	LIVER	DUODENUM
JEJUNUM	ILEUM	CECUM
COLON	THYROID GL	ADRENAL GL
SPLEEN	LYMPH ND, MES	THYMIC REGION
BONE MARROW	BONE, STERNUM	BRAIN
SPINAL CORD	NERVE, SCIATIC	TESTES
EPIDIDYMIDES	SEMINAL VESICLE	COAGULATING GL
PROSTATE	NOSE/TURBINATES	TRACHEA
KIDNEYS	URINARY BLADDER	

ANIMAL ID: 502 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
SALIVARY GL (microscopic)		
	VACUOLATION OF ACINAR AND DUCT CELLS, multifocal, minimal	
STOMACH (microscopic)		
	EDEMA, focal, minimal	
	GASTRITIS, focal, minimal	
CECUM (microscopic)		
	LYMPHOID HYPERPLASIA, multifocal, mild	
	EDEMA, focal, minimal	
LYMPH ND, CERVICAL (microscopic)		
	LYMPHOID HYPERPLASIA, multifocal, mild	

Animal 502 (continued)

TABLE 3
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
INDIVIDUAL ANIMAL DIAGNOSES FOR MALE RATS

Group: 0 mg/L

ANIMAL ID: 502 (continued)

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
PROSTATE (microscopic)		
	PROSTATITIS, multifocal, mild	
LUNGS (microscopic)		
	ATELECTASIS, multifocal, minimal	
	OSSIFICATION, METAPLASTIC, focal, minimal	
	MINERALIZATION, PULMONARY VESSEL(S), multifocal, minimal	

The following tissues/anatomic sites are microscopically normal:

HEART	LIVER	DUODENUM
JEJUNUM	ILEUM	COLON
THYROID GL	ADRENAL GL	SPLEEN
LYMPH ND, MES	THYMIC REGION	BONE MARROW
BONE, STERNUM	BRAIN	SPINAL CORD
NERVE, SCIATIC	TESTES	EPIDIDYMIDES
SEMINAL VESICLE	COAGULATING GL	NOSE/TURBINATES
LARYNX	TRACHEA	KIDNEYS
URINARY BLADDER		

ANIMAL ID: 503 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
SALIVARY GL (microscopic)		
	VACUOLATION OF ACINAR AND DUCT CELLS, multifocal, minimal	
STOMACH (microscopic)		
	GLAND ECTASIA, multifocal, mild	
	GASTRITIS, multifocal, minimal	
LIVER (microscopic)		
	TRIADITIS, multifocal, minimal	
THYMIC REGION (gross)		
	HEMORRHAGE	
	Comment: 2+.	
THYMIC REGION (microscopic)		
	LYMPHOCYTE DEGENERATION, multifocal, minimal	
EPIDIDYMIDES (microscopic)		
	EPIDIDYMITIS, focal, mild	
	DEGENERATING CELLS WITHIN TUBULE LUMENS, focal, minimal	
PROSTATE (microscopic)		
	PROSTATITIS, multifocal, mild	
	GLAND ECTASIA, multifocal, mild	

Animal 503 (continued)

TABLE 3
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
INDIVIDUAL ANIMAL DIAGNOSES FOR MALE RATS

Group: 0 mg/L

ANIMAL ID: 503 (continued)

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
LARYNX (microscopic)		
	LARYNGITIS, multifocal, minimal	
LUNGS (microscopic)		
	MINERALIZATION, PULMONARY VESSEL(S), multifocal, minimal	
	ATELECTASIS, multifocal, minimal	

The following tissues/anatomic sites are microscopically normal:

HEART	DUODENUM	JEJUNUM
ILEUM	CECUM	COLON
THYROID GL	ADRENAL GL	SPLEEN
LYMPH ND, CERVICAL	LYMPH ND, MES	BONE MARROW
BONE, STERNUM	BRAIN	SPINAL CORD
NERVE, SCIATIC	TESTES	SEMINAL VESICLE
COAGULATING GL	NOSE/TURBINATES	TRACHEA
KIDNEYS	URINARY BLADDER	

ANIMAL ID: 504 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
SALIVARY GL (microscopic)		
	VACUOLATION OF ACINAR AND DUCT CELLS, multifocal, minimal	
ILEUM (microscopic)		
	LYMPHOID HYPERPLASIA, focal, mild	
THYMIC REGION (microscopic)		
	LYMPHOCYTE DEGENERATION, multifocal, minimal	
PROSTATE (microscopic)		
	PROSTATITIS, multifocal, moderate	
LARYNX (microscopic)		
	LARYNGITIS, multifocal, minimal	
LUNGS (microscopic)		
	ALVEOLAR HISTIOCYTOSIS, multifocal, minimal	
	ATELECTASIS, multifocal, minimal	
URINARY BLADDER (microscopic)		
	EDEMA, focal, mild	

Animal 504 (continued)

TABLE 3
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
INDIVIDUAL ANIMAL DIAGNOSES FOR MALE RATS

Group: 0 mg/L

ANIMAL ID: 504 (continued)

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE

The following tissues/anatomic sites are microscopically normal:

HEART	STOMACH	LIVER
DUODENUM	JEJUNUM	CECUM
COLON	THYROID GL	ADRENAL GL
SPLEEN	LYMPH ND, CERVICAL	LYMPH ND, MES
BONE MARROW	BONE, STERNUM	BRAIN
SPINAL CORD	NERVE, SCIATIC	TESTES
EPIDIDYMIDES	SEMINAL VESICLE	COAGULATING GL
NOSE/TURBINATES	TRACHEA	KIDNEYS

ANIMAL ID: 505 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE

SALIVARY GL (microscopic)
VACUOLATION OF ACINAR AND DUCT CELLS, multifocal, minimal
STOMACH (microscopic)
GLAND ECTASIA, multifocal, minimal
GASTRITIS, multifocal, minimal
ILEUM (microscopic)
LYMPHOID HYPERPLASIA, focal, mild
LYMPH ND, CERVICAL (microscopic)
LYMPHOID HYPERPLASIA, diffuse, marked
LUNGS (microscopic)
ATELECTASIS, focal, minimal

The following tissues/anatomic sites are microscopically normal:

HEART	LIVER	DUODENUM
JEJUNUM	CECUM	COLON
THYROID GL	ADRENAL GL	SPLEEN
LYMPH ND, MES	THYMIC REGION	BONE MARROW
BONE, STERNUM	BRAIN	SPINAL CORD
NERVE, SCIATIC	TESTES	EPIDIDYMIDES
SEMINAL VESICLE	COAGULATING GL	PROSTATE
NOSE/TURBINATES	LARYNX	TRACHEA
KIDNEYS	URINARY BLADDER	

TABLE 3
A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT
INDIVIDUAL ANIMAL DIAGNOSES FOR MALE RATS

Group: 0.1 mg/L

ANIMAL ID: 506 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
HEART (microscopic)		
	MYOCYTE VACUOLATION, multifocal, minimal	
	MYOCARDITIS, multifocal, minimal	
	MUSCLE FIBER DEGENERATION, multifocal, mild	
LIVER (microscopic)		
	HEPATOCELLULAR CYTOPLASMIC VACUOLATION, multifocal, mild	
	BILE DUCT HYPERPLASIA, multifocal, minimal	
	TRIADITIS, multifocal, minimal	
LYMPH ND, CERVICAL (gross)		
	ENLARGED	G-01
	Comment: 3+.	
LYMPH ND, CERVICAL (microscopic)		
	LYMPHOID HYPERPLASIA, focal, marked	M-01

The following tissues/anatomic sites are microscopically normal:
TESTES EPIDIDYMIDES

The two digit number correlates one or more microscopic findings (M-) with one or more gross findings (G-)

ANIMAL ID: 507 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
HEART (microscopic)		
	MYOCARDITIS, multifocal, minimal	

The following tissues/anatomic sites are microscopically normal:
LIVER TESTES EPIDIDYMIDES

ANIMAL ID: 508 DATE OF DEATH:16-JUL-97 MALE SCHEDULED SACRIFICE

TISSUE/ANATOMIC SITE	DIAGNOSES	GROSS/MICRO CORRELATE
HEART (microscopic)		
	MYOCARDITIS, multifocal, mild	
	MUSCLE FIBER DEGENERATION, multifocal, mild	
LIVER (microscopic)		

Animal 508 (continued)